ARCHIVES

OF

THE MIDDLESEX HOSPITAL

VOLUME XXVII.

Eleventh Report

FROM THE

Cancer Research Laboratories

CONTENTS.

London

MACMILLAN AND CO., LIMITED

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Eleventh Report

FROM THE

Cancer Research Laboratories

(Being the Twenty-seventh Volume of the Archives)

EDITED FOR THE CANCER INVESTIGATION COMMITTEE

BY

W. S. LAZARUS-BARLOW, M.D., F.R.C.P.,

DIRECTOR OF THE CANCER RESEARCH LABORATORIES; LECTURER ON GENERAL PATHOLOGY AT THE MIDDLESEX HOSPITAL; FOREIGN MEMBER OF THE GERMAN COMMITTEE FOR THE INVESTIGATION OF CANCER; FORMERLY PATHOLOGIST AND LECTURER ON PATHOLOGY AT THE WESTMINSTER HOSPITAL

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NOTICE.

In the following Pages, excepting where an asterisk (*) *is placed, or where the context makes it clear that such is not the case,* EVERY DIAGNOSIS OF MALIGNANT DISEASE HAS BEEN MADE AS THE RESULT OF MICROSCOPICAL EXAMINATION.

W. S. L.-B.

REPORTS

FROM THE

CANCER RESEARCH LABORATORIES

TABULATED SYNOPSES OF THE POST-MORTEM EXAMINATIONS AND OPERATIONS IN CASES OF MALIGNANT DISEASE DURING THE YEAR 1911.

By THE DIRECTOR and his ASSISTANTS

In the following tables are given the results of all cases of malignant disease, as determined by microscopical examination, which were investigated in the Cancer Research Laboratories during the year. The material was derived partly from the post-mortem room and partly from the operating theatre. In all 191 cases of malignant disease (males 62, females 129) have been examined microscopically. All of the above were in-patients. The total number of admissions to the hospital as in-patients during 1911 was 3,815;* viz., 1,835 males, and 1,980 females. In addition 15 males and 27 females with malignant disease were admitted to the electrical (out-patient) department for X-ray treatment. Histological examination was not made in these cases.

Besides the cases that have been mentioned, a certain number of patients were admitted (either to the general

* In addition to the above there were 361 admissions to the special maternity wards, and 322 babies were born in hospital.

wards or to the special wards) in which the diagnosis was not made certain by histological examination. These are grouped in two classes according to the relative probability of accuracy in the diagnosis.

In the first group, the diagnosis was founded on naked-eye appearances or on touch, but the patients were either discharged unrelieved from the Hospital at their own request, or else left after palliative or exploratory operation (*e.g.*, cases of gastrostomy, colotomy, &c.).

In the second group the diagnosis was made upon clinical grounds alone.

GROUP I.

Cases diagnosed as Malignant Disease on Evidence derived from the Naked-Eye Appearance, or Touch.

Site.	1911.		
	Males.	Females.	Total.
Uterus	—	40	40
Breast	—	42	42
Breast and Rectum	—	1	1
Rectum	17	14	31
Ovary	—	3	3
Bladder	2	3	5
Tongue	8	—	8
Lip	1	—	1
Mouth	2	—	2
Pharynx	1	1	2
Larynx	4	—	4
Penis	2	—	2
Kidney	2	—	2
Vulva	—	3	3
Sup. Maxilla	—	2	2
Anal Canal	—	1	1
Cheek	3	—	3
Prostate	3	—	3
Tonsil	1	1	2
Face	—	1	1
Palate	1	—	1
Hand	1	—	1
Glands in Neck	1	—	1
Totals	49	112	161

GROUP II.

Cases diagnosed on Clinical Evidence only.

Site.	1911.		
	Males.	Females.	Total.
Stomach	18	7	25
Œsophagus	14	3	17
Colon	2	6	8
Gall-bladder	2	1	3
Cæcum	1	1	2
Intestine	—	2	2
Liver	1	—	1
Totals	38	20	58

TABLE I.—

	Total.		0—5.		6—10.		11—15.		16—20.		21—25.		26—30.		31—35.	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
CARCINOMA.																
Face ... ... Squamous	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tongue ... ... Squamous	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tonsil ... ... Squamous	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Œsophagus ... ... Squamous	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Stomach, Body ... Spheroidal	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
„ Pylorus ... Spheroidal	1	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—
„ Pylorus ... Columnar	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cæcum ... ... Spheroidal	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Transverse Colon ... Columnar	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Splenic Flexure ... Spheroidal	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sigmoid ... ... Columnar	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rectum ... ... Columnar	2	6	—	—	—	—	—	—	—	—	—	1	—	—	—	—
Rectum ... ... Spheroidal	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Anus ... ... Squamous	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Liver ... ... Spheroidal	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gall-bladder ... ... Columnar	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pancreas ... ... Spheroidal	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Urinary bladder ... Squamous	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Testicle ... ... Spheroidal	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
... ... Columnar	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
... ... Spheroidal	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cervix Uteri ... ... Squamous	—	5	—	—	—	—	—	—	—	—	—	—	—	1	—	—
Cervix Uteri ... ... Columnar	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vulva ... ... Squamous	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Breast ... ... Spheroidal	—	9	—	—	—	—	—	—	—	—	—	—	—	—	..	..
Breast ... ... Columnar	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SARCOMA.																
Lympho-sarcoma	—	2	—	—	—	—	—	—	—	cervical glands	—	—	—	—	—	—
Small round-cell	1	—	—	—	—	—	—	—	—	—	peritoneum	—	—	—	—	—
Melanotic	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DOUBTFUL	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
ENDOTHELIOMA	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	27	41														

Post-mortem Cases.

36—40.		41—45.		46—50.		51—55.		56—60.		61—65.		66—70.		71—75.		76—80.		81—85.		86—90.	
M.	F.	M.	F.	M.	F.	M.	F	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
—	—	—	—	2	—	—	—	—	—	2	—	—	—	1	—	—	—	—	—	—	—
—	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	1	1	—	—	2	—	—	—	1	1	1	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	—	—
—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	1	—	1	—	1	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	1	—	3	—	2	—	1	—	1	—	—	—	—	—	—	—	1
—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	Media-stinum	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	skin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	breast	—	—	—	—	—	ovary	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	gall-bladder	small intes-tine.	breast	—	—	—	—	rectum	—	—	—	—	—	—	—

TABLE II.—

	Total.		0—5.		6—10.		11—15.		16—20.		21—25.		26—30.		31—35.	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
CARCINOMA.																
Lip ... ... Squamous	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Tongue ... ... Squamous	12	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Thyroid ... ... Spheroidal	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Intestine ... ... Columnar	6	3	—	—	—	—	—	—	—	—	—	—	rectum	—	—	—
Uro-genital System ... Squamous	2	15	—	—	—	—	—	—	—	—	—	—	—	cervix uteri	—	cervix uteri
Uro-genital System ... Columnar	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Uro-genital System ... Papilliferous Cystadenoma	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Breast ... ... Columnar	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Breast ... ... Spheroidal	—	41	—	—	—	—	—	—	—	—	—	—	—	—	—	1
Other Sites ... Squamous	5	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
SARCOMA.																
Small round-cell	—	2	—	—	—	—	—	—	—	—	—	orbit	—	—	—	—
Spindle-cell	1	—	—	—	—	—	—	—	—	—	—	—	ilium	—	—	—
Myeloid	—	1	—	—	—	—	—	—	—	—	—	—	—	jaw	—	—
Melanotic	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Myxosarcoma	1	—	—	—	—	—	—	—	—	femur	—	—	—	—	—	—
CHORIONEPITHELIOMA ...	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1
ENDOTHELIOMA	2	6	—	—	—	—	—	—	—	—	—	—	—	—	—	breast 2
DOUBTFUL	2	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	35	88	TOTAL NUMBER OF HOSPITAL OPERATION CASES FOR MALIGNANT DISEASE										Carcinoma— Males 28, Females 70 Sarcoma „ 3, „ 3 Chorionepithelioma „ 0, „ 1 Endothelioma „ 2, „ 6 Doubtful „ 2, „ 8			
NON-MALIGNANT.	22	44	—	—	—	—	1	1	2	—	1	2	—	5	3	4
	57	132	TOTAL NUMBER OF HOSPITAL OPERATION CASES EXAMINED IN THE CANCER RESEARCH LABORATORIES.													

Operation Cases.

36—40.		41—45.		46—50.		51—55.		56—60.		61—65.		66—70.		71—75.		76—80.	
M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
—	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—
1	—	2	—	1	1	3	—	4	—	—	—	—	—	1	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
—	—	—	—	rectum	rectum	rectum	sigmoid (2)	—	—	rectum sigmoid	—	—	—	—	—	sigmoid	—
penis	cervix uteri	penis	cervix uteri	—	cervix uteri (4)	—	cervix uteri	—	cervix uteri (3)	—	cervix uteri	—	vulva	—	—	—	vulva
—	—	—	—	—	—	—	corpus uteri ovary	—	—	—	—	—	—	—	—	—	—
—	—	—	1	—	—	—	1	—	1	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—
—	5	—	8	—	6	—	2	—	9	—	4	—	5	—	1	—	—
—	skin	—	—	skin (2)	—	skin —	skin (2)	—	—	—	—	skin	—	skin	—	—	—
—	ovary	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	foot	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	breast (2)	perito-neum	breast	—	breast	—	—	parotid	—	—	—	—	—	—	—	—	—
—	—	—	arm cervix uteri	—	—	—	breast (2) cervix uteri pelvis	thigh	breast	—	—	—	—	jaw	—	—	face
—	6	2	8	2	6	2	6	5	2	—	2	4	1	—	1	—	—

TABLE III.

Synopses of Post-mortem Cases.

CARCINOMATA.

No	Initials and Cancer Register Number.	Sex.	Age at Death.	Nature of new growth and part primarily affected.	Sites of Secondary new growth.	Other morbid changes present.	(i.) Congenital abnormalities. (ii.) General remarks.	(i.) Date of admission. (ii.) Date of death. (iii.) Surgical operation, if any.
1	W.H. 244/11	M	57	Squamous cell carcinoma of face.	Submaxillary glands.	... ...	(ii) Fair nutrition.	(i) 28 Oct. '11. (ii) 23 Nov. '11.
2	C.T. 261/11	F	60	Squamous cell carcinoma of face.	Cervical glands.	... ...	(ii) Fair nutrition.	(i) 3 Aug. '11. (ii) 11 Dec. '11.
3	R.E. 121/11	M	50	Squamous cell carcinoma of tongue.	Submaxillary glands.	Pulmonary tuberculosis.	(ii) Emaciated.	(i) 25 Jan. '11. (ii) 25 May '11.
4	T.F. 74/11	M	59	Squamous cell carcinoma of tongue.	Thyroid, submaxillary glands.	Pulmonary tuberculosis. Fracture of mandible.	(ii) Emaciated.	(i) 21 Dec. '10. (ii) 29 March '11.
5	T.S. 15/11	M	80	Squamous cell carcinoma of left tonsil.	Submaxillary glands.	Right empyema	(ii) Slight emaciation.	(i) 2 Jan. '11. (ii) 14 Jan. '11.
6	B.W. 190/11	M	48	Squamous cell carcinoma of œsophagus, middle third.	Bronchial and cervical glands.	... ...	(ii) Emaciated. Hosp. p.m. 149.	(i) 26 Aug. '11. (ii) 2 Sept. '11
7	W.J. 133/11	M	63	Squamous cell carcinoma of œsophagus. middle third.	None.	... ...	(ii) Emaciated. Hosp. p.m. 114.	(i) 29 April '11. (ii) 12 June '11.
8	W.T. 39/11	M	63	Squamous cell carcinoma of œsophagus, middle third.	None.	... ...	(ii) Thin. Hosp. p.m. 31.	(i) 4 Feb. '11. (ii) 6 Feb. '11. (iii) Gastrostomy

9	J.G. 173/11	M	50	Squamous cell carcinoma of œsophagus, lower end.	Stomach, bronchial glands.	Old pleural adhesions.	(ii) Emaciated.	(i) 27 Feb. '11. (ii) 10 Aug. '11.
10	M.B. 108/11	M	72	Squamous cell carcinoma of lower end of œsophagus.	None.	... ...	(ii) Emaciated.	(i) 13 March '11. (ii) 3 May '11. (iii) Gastrostomy.
11	H.A. 135/11	M	43	Spheroidal cell carcinoma of body of stomach.	Bronchial glands.	... ...	Hosp. p.m. (*Notes lost.*)	(i) 10 May '11. (ii) 14 June '11.
12	R.W. 226/11	M	59	Spheroidal cell carcinoma of body of stomach	Diaphragm, portal gland.	Double pleural effusion.	(ii) Emaciated. Hosp. p.m. 179.	(i) 18 Oct. '11. (ii) 3 Nov. '11.
13	A.R. 218/11	M	31	Spheroidal cell carcinoma of pylorus.	Liver, mesenteric glands.	... ...	(ii) Very emaciated. Hosp. p.m. 172.	(i) 17 Sept. '11. (ii) 17 Oct. '11.
14	E.J. 147/11	M	48	Columnar cell carcinoma of pylorus.	None.	Local peritonitis.	(ii) Poor nutrition. Hosp. p.m. 130.	(i) 12 July '11. (ii) 12 July '11. (iii) Gastro-enterostomy.
15	M.W. 91/11	M	50	Columnar cell carcinoma of pylorus.	Portal gland.	... ...	(ii) Emaciated. Hosp. p.m. 77.	(i) 6 April '11. (ii) 17 April '11.
16	E.A. 127/11	F	55	Spheroidal cell carcinoma of pylorus.	Liver.	... ...	Hosp. p.m. (*Notes lost.*)	(i) 13 May '11. (ii) 1 June '11.
17	E.W. 136/11	F	55	Colloid spheroidal cell carcinoma of cæcum.	Mesenteric glands liver.	... ...	(ii) Nutrition fair. Hosp. p.m. 118.	(i) 27 May '11. (ii) 17 June '11.
18	W.D. 1/11	M	60	Spheroidal cell carcinoma of cæcum.	Liver, peritoneum.	... ...	(ii) Great emaciation. Hosp. p.m. 2.	(i) 13 Dec. '10. (ii) 2 Jan. '11.
19	A.D. 51/11	M	36	Columnar cell carcinoma of transverse colon.	Liver.	Thrombosis of right pulmonary artery. General peritonitis.	Hosp. p.m. 44.	(i) 15 Feb. '11. (ii) 18 Feb. '11. (iii) Intestinal anastomosis.

Table III.—Synopses of Post-mortem Cases—*cont.*

No.	Initials and Cancer Register Number.	Sex.	Age at Death.	Nature of new growth and part primarily affected.	Sites of secondary new growth.	Other morbid changes present.	(i.) Congenital abnormalities. (ii.) General remarks.	(i.) Date of admission. (ii.) Date of death. (iii.) Surgical operation, if any.
20	M.J. 272/11	F	66	Columnar cell carcinoma of transverse colon.	None.	... ...	(ii) Body stout. Hosp. p.m. 200.	(i) 1 Dec. '11. (ii) 20 Dec. '11.
21	A.M. 113/11	M	60	Spheroidal cell carcinoma of splenic flexure.	Liver.	... ...	Hosp. p.m.	(i) 10 May '11. (ii) 12 May '11.
22	A.P. 84/11	F	41	Columnar cell carcinoma of sigmoid.	Stomach, pancreas, liver, spleen, adrenals, bladder, pleura, peritoneum, pre-aortic and œsophageal glands.	Ascites, double pleural effusion.	(ii) Emaciated.	(i) 31 March '11. (ii) 5 April '11.
23	L.A. 145/11	F	21	Columnar cell carcinoma of rectum.	Liver.	Pulmonary tuberculosis, tuberculous ulcers of whole of large intestine.	(ii) Emaciated.	(i) 23 March '11. (ii) 11 July '11.
24	J.L. 62/11	M	36	Spheroidal cell carcinoma of rectum (colloid).	Skin, prostate, small intestine, stomach; aortic and iliac glands.	Double hydronephrosis.	(ii) Emaciated.	(i) 27 Jan. '11. (ii) 10 March '11.
25	R.F. 73/11	F	50	Columnar cell carcinoma of rectum.	None.	... ...	(ii) Well nourished.	(i) 18 Feb. '11. (ii) 27 March '11.
26	J.B. 83/11	M	55	Columnar cell carcinoma of rectum.	None.	... ...	(ii) Well nourished. Hosp. p.m.	(i) 18 March '11. (ii) 4 April '11. (iii) Laparotomy, left inguinal colotomy.
27	S.T. 9/11	F	58	Columnar cell carcinoma of rectum.	None.	Œdema of left leg.	(ii) Emaciated.	(i) 23 Dec. '10. (ii) 6 Jan. '11.

28	F.B 175/11.	F	58	Columnar cell carcinoma of rectum.	None.	Old pleural adhesions, gallstones.	(ii) Emaciated.	(i) 8 Feb. '11. (ii) 15 Aug. '11. (iii) Laparotomy, left inguinal colotomy.
29	G.C. 184/11	F	70	Columnar cell carcinoma of rectum.	Fallopian tube, lumbar glands.	Old pleural adhesions. General peritonitis. Gallstone.	(ii) Emaciated.	(i) 2 March '11. (ii) 24 Aug. '11.
30	L.S. 118/11	M	71	Columnar cell carcinoma of rectum.	None.	Bronchopneumonia. Double hydronephrosis.	(ii) Very emaciated.	(i) 20 Nov. '06. (ii) 20 May '11.
31	A.R. 129/11	F	74	Columnar cell carcinoma of rectum.	Liver.	... ...	(ii) Well nourished.	(i) 10 Dec. '10. (ii) 3 June '11.
32	E.K. 109/11	F	68	Squamous cell carcinoma of anus.	Lung.	... ...	(ii) Emaciated.	(i) 17 Jan. '11. (ii) 6 May '11.
33	E.R. 196/11	F	81	Squamous cell carcinoma of anus.	Inguinal glands.	Right pleural effusion. Gallstones. Renal calculus.	(ii) Emaciated.	(i) 22 Aug. '11. (ii) 6 Sept. '11.
34	W.W. 35/11	M	55	Spheroidal cell carcinoma of liver (with cirrhosis).	Aortic stenosis and regurgitation.	Jaundice, ascites.	(ii) Well nourished. Hosp. p.m. 24.	(i) 7 Jan. '11. (ii) 1 Feb. '11.
35	E.N. 92/11	F	70	Columnar cell carcinoma of gall-bladder.	Lung, pleura, liver, small intestine.	Gallstones.	(ii) Emaciated. Hosp. p.m. 79.	(i) 8 Feb. '11. (ii) 18 April '11.
36	M.K. 195/11	F	61	Spheroidal cell carcinoma of pancreas.	Omentum.	Ascites, jaundice.	(ii) Emaciated. Hosp. p.m. 151.	(i) 31 Aug. '11. (ii) 7 Sept. '11.
37	R.C. 137/11	F	70	Spheroidal cell carcinoma of pancreas.	Thyroid.	... ...	Hosp. p.m. (*Notes lost.*)	(i) 19 April '11. (ii) 16 June '11.
38	J.T. 68/11	M	37	Squamous cell carcinoma of urinary bladder.	None.	Double pyonephrosis.	(i) Meckel's diverticulum. (ii) Slight emaciation Hosp. p.m. 54.	(i) 28 Jan. '11. (ii) 21 March '11.

Table III.—Synopses of Post-mortem Cases—*cont.*

No.	Initials and Cancer Register Number.	Sex.	Age at Death.	Nature of new growth and part primarily affected.	Sites of Secondary new growth.	Other morbid changes present.	(i.) Congenital abnormalities. (ii.) General remarks.	(i.) Date of admission. (ii.) Date of death. (iii.) Surgical operation, if any.
39	W.M. 266/11	M	49	Spheroidal cell carcinoma of testicle.	Lung, lumbar glands.	... ...	(ii) Well nourished. Hosp. p.m. 199.	(i) 13 Dec. '11. (ii) 16 Dec. '11.
40	M.S. 32/11	F	36	Columnar cell carcinoma of ovary.	None.	General peritonitis. Right hydronephrosis.	(ii) Well nourished. Hosp. p.m. 22.	(i) 21 Jan. '11. (ii) 30 Jan. '11. (iii) Laparotomy.
41	B.V. 2/11	F	60	Spheroidal cell carcinoma of ovary.	Uterus, diaphragm, liver.	Intestinal obstruction. Stercoral ulceration. Perforation. Peritonitis.	(ii) Well nourished. Hosp. p.m. 3.	(i) 31 Dec. '10. (ii) 3 Jan. '11.
42	L.C. 177/11	F	28	Squamous cell carcinoma of cervix uteri.	None.	Old pleural adhesions. Double hydronephrosis. Vesico-vaginal fistula. Cystitis.	(ii) Very emaciated.	(i) 2 Aug. '11. (ii) 16 Aug. '11.
43	E.J. 38/11	F	40	Squamous cell carcinoma of cervix uteri.	Lumbar glands.	Right hydronephrosis.	(ii) Fair nutrition.	(i) 17 Nov. '10. (ii) 2 Feb. '11.
44	E.M. 164/11	F	45	Squamous cell carcinoma of cervix uteri.	None.	Double hydronephrosis.	(ii) Well nourished.	(i) 29 June '11. (ii) 4 Aug '11. (iii) Exploratory laparotomy.
45	M.C. 149/11	F	46	Squamous cell carcinoma of cervix uteri.	Liver, lumbar glands.	Gallstones.	(ii) Well nourished.	(i) 13 April '11. (ii) 17 July '11. (iii) Abdominal hysterectomy.
46	J.B. 8/11	F	62	Columnar cell carcinoma of uterus.	Pelvic wall, lung.	... ...	(ii) Well nourished. Hosp. p.m. 6.	(i) 4 Jan. '11. (ii) 6 Jan. '11. (iii) Wertheim's hysterectomy

47	J.S. 90/11	F	69	Squamous cell carcinoma of cervix uteri.	Lumbar glands.	Peritonitis.	(ii) Fair nutrition.	(i) 18 May '08. (ii) 11 April '11.
48	M.S. 253/11	F	64	Squamous cell carcinoma of vulva.	Liver, spleen, intestine, uterus, Fallopian tube, heart, pericardium, peritoneum; lumbar, portal, and bronchial glands.	Chronic peritonitis.	(ii) Emaciated.	(i) 13 Oct. '11. (ii) 2 Dec. '11.
49	S.P. 18/11	F	49	Spheroidal cell carcinoma of right breast.	Left breast, liver, lungs, thyroid, stomach, pancreas, appendix, pleura; axillary, supraclavicular, and mesenteric glands.	... ...	(ii) Fair nutrition.	(i) 12 Aug. '10. (ii) 14 Feb. '11.
50	A.B. 243/11	F	51	Spheroidal cell carcinoma of left breast.	Lung, liver; axillary, bronchial, and mesenteric glands.	Double pleural effusion. Œdema of left arm.	(i) Absence of right kidney. (ii) Emaciated.	(i) 1 Aug. '11. (ii) 20 Nov. '11.
51	S.W. 202/11	F	52	Spheroidal cell carcinoma of right breast.	Axillary glands; liver*.	Gallstones.	(ii) Emaciated.	(i) 28 Aug. '11. (ii) 22 Sept. '11.
52	M.T. 36/11	F	53	Spheroidal cell carcinoma of left breast.	Liver.	Jaundice.	(ii) Thin. Hosp. p.m. 25.	(i) 7 Jan. '11. (ii) 1 Feb. '11. (iii) Amputation of breast.
53	E.P. 185/11	F	58	Spheroidal cell carcinoma of left breast.	Pleuræ, adrenals, liver, thyroid; mediastinal and axillary glands.	Double pleural effusion.	(ii) Well nourished.	(i) 29 Nov. '10. (ii) 24 Aug. '11.
54	A.S. 57/11	F	59	Spheroidal cell carcinoma of breast.	Lung, mediastinal glands.	Double pleural effusion.	(ii) Emaciated	(i) 20 Aug. '10. (ii) 28 Feb. '11. (iii) Amputation of breast, 1909.

Table III.—Synopses of Post-mortem Cases—*cont.*

No.	Initials and Cancer Register Number.	Sex.	Age at Death.	Nature of new growth and part primarily affected.	Sites of Secondary new growth.	Other morbid changes present.	(i.) Congenital abnormalities. (ii.) General remarks.	(i.) Date of admission. (ii.) Date of death. (iii.) Surgical operation, if any.
55	A.G. 80/11	F	59	Villous columnar cell carcinoma of left breast.	Peritoneum.	... ...	(ii) Emaciated.	(i) 7 March '11. (ii) 31 March '11.
56	A.B. 114/11	F	64	Spheroidal cell carcinoma of left breast.	Skin.	Œdema of left arm and leg. Left pleural effusion.	(ii) Emaciated.	(i) 28 March '11. (ii) 16 May '11.
57	M.B. 69/11	F	70	Spheroidal cell carcinoma of right breast.	Left breast, liver, axillary glands.	Œdema of right arm.	(ii) Well nourished.	(i) 23 Sept. '10. (ii) 23 March '11.
58	J.N. 206/11	F	87	Spheroidal cell carcinoma of right breast.	Lungs, liver.	Old pleural adhesions.	(ii) Emaciated.	(i) 31 March '09. (ii) 5 Oct. '11.

SARCOMATA.

1	M.H. 134/11	F	16	? Lymphosarcoma of cervical glands.	None.	Bronchitis, multiple gastric ulcers, old endocarditis.	(ii) Very emaciated.	(i) 19 Sept. '10. (ii) 9 June '11.
2	J.B. 47/11	M	22	Small round cell sarcoma of peritoneum.	Omentum, diaphragm, liver.	... ...	... ...	(i) 26 Jan. '11. (ii) 13 Feb. '11.
3	N.S. 138/11	F	41	Lymphosarcoma of mediastinum.	Pancreas.	Duodenal ulcer.	(ii) Thin. Hosp. p.m. 120.	(i) 10 May '11. (ii) 19 June '11.
4	W.B. 88/11	M	55	Melanotic sarcoma of skin.	Liver, bladder, peritoneum, mesenteric glands.	... ...	(ii) Emaciated. Hosp. p.m. 72.	(ii) 10 April '11.

ENDOTHELIOMATA.

1	M.K. 61/11	F	54	Endothelioma of gall-bladder.	Liver.	Gallstones.	(ii) Emaciated.	(i) 9 Jan. '11. (ii) 4 March '11.
2	J.B. 186/11	M	56	*Endothelioma of small intestine. Squamous cell carcinoma of tongue.	Mesenteric glands.	Bronchopneumonia	(ii) Thin. Hosp. p.m.	(i) 3 Aug. '11. (ii) 24 Aug. '11. (iii) Excision of part of mandible.
3	L.T. 194/11	F	60	Endothelioma of right breast.	Left breast, pleuræ, mediastinum, skull; cervical, axillary, bronchial, and lumbar glands.	Right pleural effusion, gallstones.	(ii) Well nourished.	(i) 13 June '11. (ii) 5 Sept. '11.
4	J.R. 176/11	M	72	Endothelioma of rectum.	Liver, mesenteric and lumbar glands.	Jaundice.	(ii) Well nourished.	(i) 8 July '11. (ii) 17 Aug. '11.

* Double primary.

DOUBTFUL.

1	A.B. 220/11	F	50	Malignant disease of left breast (nature doubtful).	Right breast, liver, skull, pleuræ, lung; axillary, cervical, supraclavicular glands.	Double pleural effusion.	(ii) Emaciated.	(i) 26 Oct. '11. (ii) 29 Oct. '11.
2	M.W. 183/11	F	61	Malignant disease of ovary (nature doubtful).	Pleura, peritoneum, iliac glands.	Ascites, left pleural effusion, left pyonephrosis.	(ii) Emaciated.	(i) 11 Aug. '11. (ii) 22 Aug. 11. (iii) Laparotomy.

A COMPARISON OF X-RAY AND RADIUM MEASUREMENTS FOR BIOLOGICAL PURPOSES.

By S. RUSS.

(*Communicated to the Röntgen Society, January* 2, 1912.)

It is desirable for clinical and experimental biological purposes to have some comparative measurements between the intensity and character of the radiation from an X-ray bulb working under specified conditions, and that from a known quantity of radium.

The character of the radiation from both is complex. Under whatever conditions an X-ray bulb is working, the radiation coming away from it is heterogeneous, consisting of rays varying in their penetrating power. The radiation from radium (and the short-lived products in equilibrium with it, Ra Emanation, RaA, RaB, and RaC) consists of alpha, beta, and gamma rays, which are also heterogeneous in type.

Measurements have been made of the penetrability through aluminium of the X-rays emitted by a bulb under varying conditions of spark-gap and compared with the penetrating power of beta and gamma rays through the same substance.

The alpha rays from radium are completely stopped by

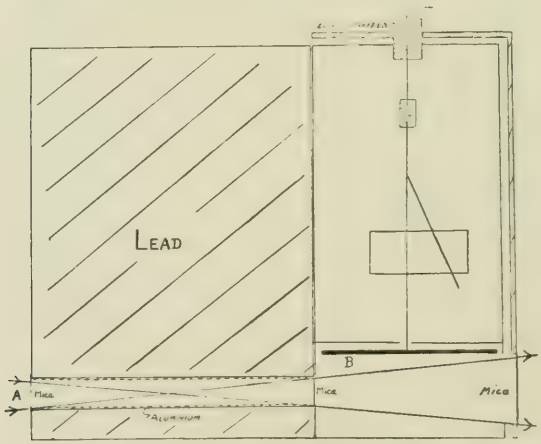


FIG. 1.

·05 mm. aluminium, so that under most conditions of irradiation their effects may be neglected.

The intensity of X-rays has been measured by the ionisation that they produce in the air of an electroscope. This has been compared with the ionisation produced in the same electroscope by the gamma rays from a known quantity of radium.

The last part of the paper affords some evidence that the biological effects of X, beta, and gamma rays are proportional to the ionising action of these rays.

Method of Measurement.

The character of the X-rays from a bulb under varying conditions of spark-gap was determined by means of the apparatus in Fig 1.

A beam of X-rays enters the electroscope through an aperture A in a lead block which was lined with aluminium to eliminate any secondary X-rays from the lead. The beam, on entering the electroscope, ionised the air beneath the metal plate B, which was connected to a gold-leaf system, the rate of fall of the gold leaf serving as a measure of the amount of ionisation produced by the rays. The mica windows allowed of the accurate alignment of the anode.

By placing aluminium screens at A in the path of the beam, the penetrability of the rays was found by the change in the ionisation thereby produced.

The criterion for the homogeneity of X-rays is that they are absorbed according to an exponential law. Corresponding to any thickness of matter traversed by the rays the ionisation has a certain value. If the logarithms of the values of the ionisation are plotted as ordinates and the thickness of matter traversed as abscissæ, the points on the diagram will lie on a straight line if the absorption of the rays has followed an exponential law.

This test when applied to the radiation from an X-ray bulb reveals the heterogeneous character of the rays, as may be seen from Fig. 2.

Three typical cases are exhibited, curves I., II., and III. corresponding to spark-gaps of 4·5, 8·5, and 19 cm. respec-

tively, the same current flowing through the primary in each case. At 19 cm. spark-gap the beam is almost homogeneous

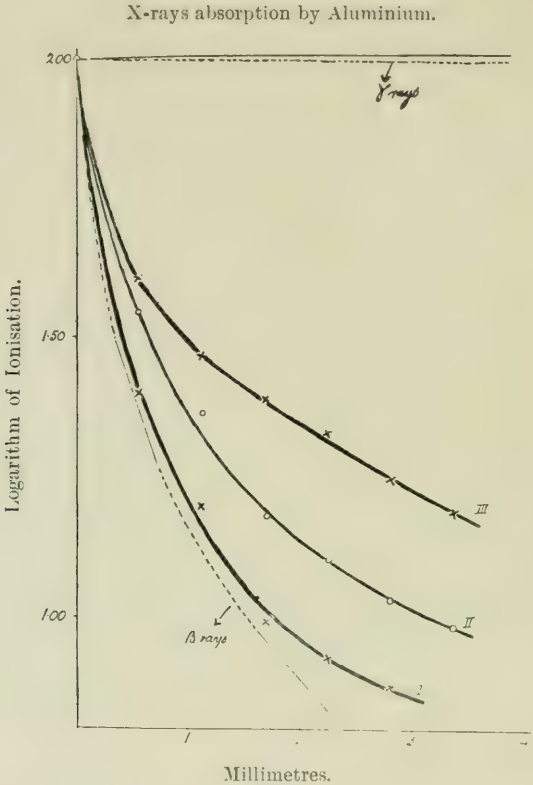


FIG. 2.

after 1·12 mm. of aluminium have been traversed, as may be seen from the approach of the curve to a straight line.

On the same diagram are drawn corresponding curves for the beta and gamma rays from radium, use being made of data obtained by Kovarik and McLelland. It will be seen that whereas the beta rays are rather more easily absorbed by aluminium than the X-rays excited by a very "soft" bulb, the gamma rays are very much more penetrating (about 30 times actually) than the X-rays from a very "hard" bulb.

The data corresponding to the three cases considered are collected in Table I.

TABLE I.

ABSORPTION OF X-RAYS BY ALUMINIUM.

Current.			
Primary.	Secondary.	Spark-gap.	
4·5 Amperes	·9 Milli-Amperes	4·5 cm.	I.
,,	·45 ,,	8·5 ,,	II.
,,	·15 ,,	19 ,,	III.

IONISATION AFTER PASSING THROUGH ALUMINIUM.

	0	·56 mm.	1·12 mm.	1·68 mm.	2·24 mm.	2·8 mm.	3·36 mm.
I.	100	25·1	15·7	9·8	8·5	7·5	—
II.	100	34·7	23·0	15·1	12·7	10·6	9·6
III.	100	40·2	29·2	24·4	21·2	17·5	15·3

PERCENTAGE ABSORBED BY ·56 MM. AL AFTER RAYS HAVE PASSED THROUGH THE FOLLOWING THICKNESSES:—

	0	·56 mm.	1·12 mm.	1·68 mm.	2·24 mm.	2·8 mm.
I.	74·9	37·4	37·6	13·2	11·2	—
II.	65·3	33·7	34·3	15·9	16·1	9·8
III.	59·8	27·2	16·4	13·1	17·4	12·5

For the whole series of observations the current through the primary coil was kept constant. If this were changed the type of radiation from the bulb would be altered.

The Coefficient of Absorption of the Rays.

It is convenient to refer to the penetrating power of a beam of rays in terms of a coefficient * (λ) the numerical value of which is inversely proportional to the penetrability of the beam. A large value of λ corresponds to an easily absorbed beam and a small value to a very penetrating one. λ also varies according to the nature of the absorbing material.

If after traversing 1 cm. of matter the ionisation in the electroscope is reduced to one half its initial value,

* The value of λ is obtained as follows:—The intensity of a homogeneous beam in its passage through matter may be represented by the equation

$$I = I_0 e^{-\lambda d}$$

where I_0 is the initial ionisation produced by the beam and I the ionisation produced after a thickness of matter d has been traversed.

Hence $\lambda = \frac{2{\cdot}302\ (\log_{10} I_0 - \log_{10} I)}{d}$

$\lambda = \cdot 69$ cm.$^{-1}$ This is just about the mean value found for the absorption by fatty tissues (breast) of a beam of X-rays of "medium" hardness (9·5 cm. spark-gap).

For a "hard" beam, previously screened by 1·12 mm. aluminium, the value of λ for the same tissue is ·45 cm.$^{-1}$ and this value is used in a later section to illustrate the fall in the intensity of X-rays in their passage through the tissues.

It is clear that when dealing with a heterogeneous beam the value of λ varies with each succeeding layer of matter traversed, whereas it is a constant if the beam is homogeneous.

Approximate values of λ or the maximum part of the radiation corresponding to cases I., II., and III. have been obtained after the very soft components were cut out. These are collected in Table II., which also contains values of λ for the beta and gamma rays from radium.

TABLE II.—VALUES OF THE COEFFICIENT OF ABSORPTION λ (cm.$^{-1}$) FOR ALUMINIUM.

	X-rays under conditions of Spark-gap.			
Beta rays	4·5 cm.	8·5 cm.	19 cm.	Gamma rays.
75—13·5	8·1	5·6	2·9	·104

The coefficient of absorption λ being inversely proportional to the penetration of the rays, the enormous difference in the penetrating power of beta and gamma rays is easily appreciated by a comparison of their coefficients.

The Ionisation caused by X and Gamma Rays.

The ionisation caused by unscreened X-rays at a spark-gap of 9 cm. and by the screened rays at a spark-gap of 19 cm. was measured and compared with that produced by the gamma rays from a known quantity of radium.

The results are collected in Table III., from which may be seen the quantity of radium required to give the same ionisation in the electroscope by means of gamma rays as that produced by an X-ray bulb working under the specified conditions.

The ionising effect in air of the beta rays from radium being about 50 times that of the gamma rays, the beta-ray equivalence may be taken as one fiftieth of the numbers given for the gamma rays.

TABLE III.—COMPARISON OF IONISATIONS.

GAMMA RAYS.

Quantity.	Distance.	Leak.	(Distance)^2 × Leak per gram Radium.
18·5 mgms. RaB_2 (emanation)	19 cm.	·51 div./min.	17,000
·357 gram $Ra\,Cl_2$	94 ,,	·54 ,, ,,	17,450
		Mean	17,225

X-RAYS.

Spark-gap.	Screen.	Distance.	Leak.	(Distance)^2 × Leak.	Gamma-ray Equivalent in grams Radium.
9 cm.	None	94 cm.	105 divs./min.	928,000	53·8
19 ,,	1·12 mm. of Al.	,,	17·3 ,, ,,	153,000	8·88

Clinical Considerations.

I.—SURFACE CONDITIONS OF IRRADIATION.

The unscreened X-rays from a bulb at a spark-gap of 9 cm. are equivalent in ionising action to the gamma rays from 53·8 grams of radium if placed in the same position as the anode (*vide* Table III.).

Experimentally the anode is not usually nearer than 10 cms. from the surface to be irradiated, whereas an application of radium may be made directly on the part in question, and therefore one-half of the total radiation is utilised. Hence, comparing the intensity of irradiation per square cm. of surface under these conditions, and taking the area of the sphere with the anode at centre as 1250 sq. cms., we have:—

X-ray intensity per sq. cm.

$= \text{gamma rays from } \frac{53{\cdot}8 \times 2}{1250} \text{ grams radium}$ per sq. cm.,

= gamma rays from 86·1 milligrams radium ,,

= gamma rays from 146·6 ,, radium bromide ,,

= beta rays from 2·93 ,, ,, ,, ,,

Assuming the simplest relation between the biological effects of the rays and the ionisation that they produce, it might be expected that the irradiation of thin layers of tissue by X-rays from a "medium" bulb or by the beta rays from

2·93 milligrams of radium bromide per sq cm., for the same interval of time, would lead to similar biological results under the conditions specified above.

Some experiments (which were communicated to the meeting of the Pathological Society of Great Britain and Ireland on January 5th, 1912) have recently been completed by Dr. B. H. Wedd and the author upon the effect of these different rays on inoculable mouse tumours (Cf. this vol., p. 50).

With an intensity of 2·81 milligrams of radium bromide per sq. cm. the effects of the beta rays upon the tumours for various times of exposure bore a marked resemblance to the effects under medium X-rays for a similar time-range.

With the above quantity of radium, an exposure of the tumours for 18 hours to the gamma rays failed to give definite evidence of any biological effects. Subsequent calculation on the above lines indicated that an exposure lasting 50 hours would have been necessary.

II.—Deep-Seated Conditions of Irradiation.

Under these circumstances a "hard" bulb would be used, and the soft constituents of the beam of X-rays may be cut out by 1·12 mm. of aluminium. Reference to Table III. shows that for a screened bulb running at 19 cm. spark-gap, the anode of which is at a distance of 10 cm. from the surface in question,

X-ray intensity per sq. cm.

$$= \text{Gamma rays from } \frac{8{\cdot}88 \times 2}{1{,}250} \text{ grams radium} \qquad \text{per sq. cm.}$$

= Gamma rays from 24·3 milligrams radium bromide „

This is the condition holding at the surface. The intensity of the X-rays in passing through the tissue diminishes owing to the increase in distance from the anode and to the absorption of the rays. The variation in intensity, due to the increase in distance, assuming the inverse square law to hold, is shown by the upper dotted line in Fig. 3, and when the absorption is taken into account the true diminution of intensity through successive layers of tissue is shown by the full line curve. The value for λ is taken as ·45 cm.$^{-1}$ (*vide supra*).

If a disc 1 cm. in diameter be covered with radium, the gamma radiation per sq. cm. vertically below it decreases with the distance in the manner indicated by the lower dotted curve in Fig. 3. Taking the absorption of the rays by the tissue into account, the full line curve is obtained.

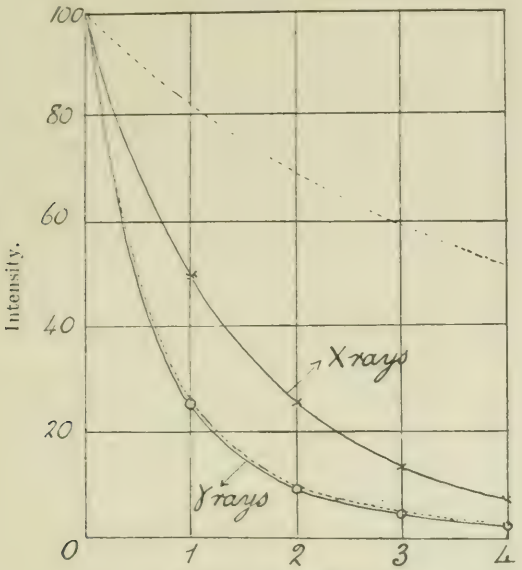


FIG. 3.

The rapid fall in the intensity through successive layers is almost entirely due to the increase in distance, the absorption by the tissue being very small.

It will be seen that starting with equivalent intensities at the surface, there would, under the experimental conditions cited, be a more marked diminution in the intensity of the gamma rays than in the X-rays, despite the fact that the absorption of the rays by the tissues was nearly 30 times greater in the latter than in the former case.

It must be understood that this quantitative estimation of the amount of radiation received by different layers of tissue is not a general one, but refers to the particular case in question. If the radium were deposited over a larger area the fall in intensity through the tissues would not be so large.

THE CLINICAL USE OF THE ACTIVE DEPOSIT OF RADIUM.

By C. R. C. LYSTER and S. RUSS.

(*Communicated to the Electro-Therapeutical Section of the Royal Society of Medicine*, vol. v., 1912, p. 150.)

It is well known that radium spontaneously produces a gas, called radium emanation. This gas, although chemically inert, has radio-active properties, and is gradually transformed into a solid substance known as radium A. In the act of transformation an alpha particle is ejected from the emanation atom, which is now an atom of RaA. This substance in turn is radio-active, emits alpha particles, and is transformed into another solid substance RaB. The transformation of this substance into RaC is not accompanied by the emission of alpha rays, but by beta and gamma rays. Radium C emits alpha, beta, and gamma rays, and is converted into another substance, RaD, which is radio-active to such a slight extent that the consideration of the radium series may be left at this stage for our present purposes. Radium C has recently been shown to be complex, consisting of two substances, RaC_1 and RaC_2, so that in speaking of RaC its dual nature must be understood.

The complex nature of the changes occurring in the sequence of events narrated is conveniently illustrated by Fig. 1, which is a representation of what is believed to occur to the atoms of the radio-active bodies in question.

The time-period is the time taken for the activity of a radio-active body to be reduced to one-half of its initial value. Thus 1 grm. of radium would by its continuous conversion into the emanation be reduced to 0·5 grm. in 1,760 years; 1 c.mm. of pure emanation would be reduced to 0·5 c.mm. in 3·86 days, owing to its transformations into RaA, and so on. The three substances, RaA, RaB, and RaC, have short time-periods, and for this reason they are collectively known as the active deposit of quick change in distinction to the bodies RaD, RaE, and RaF, which have longer periods. When a sample of radium is sealed up, the active deposit is

also present in a definite ratio. If the emanation is let into a vessel, and after being kept there some time is pumped out, the walls of the vessel are found to be radio-active: this is owing to the active deposit which has been formed from the emanation.

Under most clinical conditions of irradiation the alpha rays play no part (this is not the case, however, when fluids containing emanation in solution are injected), and the effects attendant upon irradiation are therefore due to the beta and gamma rays and the secondary rays which they produce in their passage through the tissues. Inspection of Fig. 1 shows that the beta and gamma rays from radium are almost entirely confined to a part of the active deposit (RaB and RaC): hence, by abstracting this active deposit from a sample of radium, its beta and gamma ray activity may be temporarily almost entirely withdrawn.

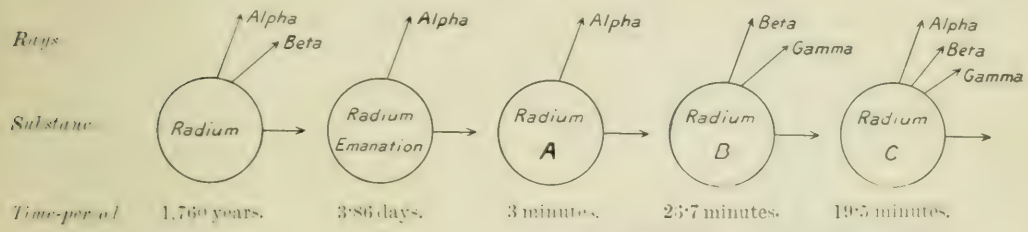


FIG. 1.

It was first shown by Rutherford* that if the emanation be let into a vessel, the active deposit, as it is formed, may be concentrated on a negative electrode and removed from the gas. The negative electrode may take any form which the clinical conditions indicate as desirable. For some time we have made use of the active deposit, upon wires for application to superficial cases, upon surgical needles for the introduction of radio-active material into growths, and upon large metal surfaces where the area to be irradiated has been extensive.

A method by which the active deposit may be concentrated from the emanation is shown in Fig 2. The case is that of a wire or needle upon which the material is to be

* Rutherford, "Philosophical Magazine," 1900, 5th ser., xlix, pp. 161–92.

deposited. The emanation is let into a small glass tube T, which is held over mercury. This tube is lined by a piece of iron gauze which dips into the mercury and through it is connected to the positive pole of a battery giving several hundreds of volts. The wire W to be made radio-active is mounted on a piece of capillary tubing so as to lie along the axis of the tube T. By letting the wire dip into a thread of mercury in the capillary tubing, connexion may be made to the negative pole of the battery. A water resistance is introduced to prevent a possible short circuit of the cells if the wire touches the gauze. It is thus seen that an electric field exists between the wire W and the gauze G, hence the active deposit is directed to the wire as it is formed from the

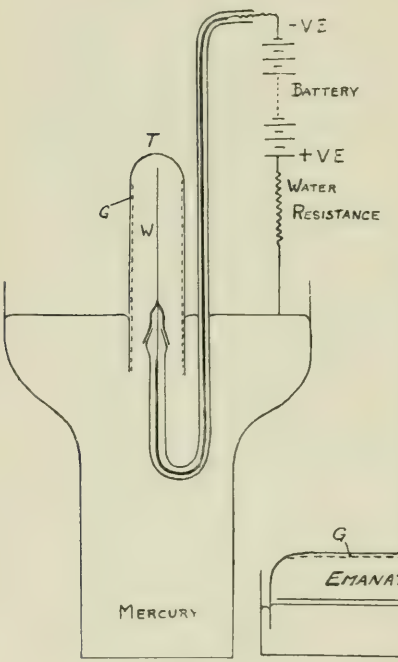


FIG. 2.

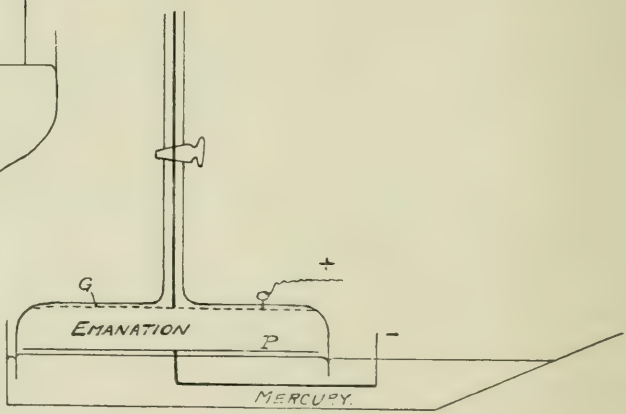


FIG. 3.

emanation. A small percentage (about 5 per cent.) goes to the gauze, but with a field of about 500 volts per centimetre nearly all of the active deposit may be obtained on the wire when dealing with moderate quantities of emanation.

Similar considerations hold for the case in which the active deposit is obtained upon the metal plate P, the method of exposure of which is shown in Fig. 3. After about three hours' exposure of the wire to the emanation the maximum quantity of active deposit is obtained upon it. Upon removal from the emanation RaA, RaB, and RaC are in radioactive equilibrium, and alpha, beta, and gamma rays are emitted by the wire. The activity of these wires is of course not permanent, but decays somewhat rapidly, the rate of decay depending upon the type of rays by which the activity is measured. This is so only because the three substances do not emit identical radiations.

The diminution with time of the gamma-ray activity is illustrated by Fig. 4, and it will be seen that after two hours the initial activity of the wire has been reduced to about 10 per cent., so that in practice irradiation of the lesion in a patient is not usually continued beyond this time. If it is desired to express the dose in terms of so much radium, the radiation from which is constant, this may be done by evaluating the area beneath the curve. To take a concrete example: Say that we start with an active wire equal in its

FIG. 4.

gamma-ray activity to 10 mgrm. of radium, then after one hour the activity of the wire is reduced to nearly 4·5 mgrm. and after two hours to about 1 mgrm. From the nature of the decay curve it is found that the total radiation for the first hour is equivalent to that from a constant supply of 7·5 mgrm., and for the two hours to a constant supply of 4·9 mgrm. This may not be the exact *therapeutic* equivalent owing to the varying intensity of the active deposit.

The advantages of this method of using radium are :—

(1) Convenience. Active wires of any shape or size may be prepared and applied with greater precision and convenience than is possible with capsules of radium.

(2) The option of using alpha and beta as well as gamma rays. For superficial conditions they are probably more effective than the latter.

(3) Economy. Several wires or needles may be exposed to the emanation at once.

The bactericidal effects of the alpha and beta rays* should ensure the sterility of the surfaces over which the radio-active material is deposited. The active deposit on these surfaces is not easily removed. An active needle after having been passed through an inch of muscular tissue was found to have lost about 30 per cent. of its gamma-ray activity.

The number of cases in which these methods have been put into practice by us is so far quite small, being confined to four cases of rodent ulcer, in which active wires having a gamma-ray activity equivalent to a few milligrammes of radium were successfully used, and to two deep-seated conditions in which surgical needles covered with active deposit were introduced.

The main purpose of this communication is not to record the results of any unique clinical manifestations as a consequence of radium treatment, but rather to indicate that the introduction of radio-active material into any desired place may be considerably simplified by the methods indicated, and that the utility of preparations of radium from which the emanation can be obtained may be considerably extended.

* Chambers and Russ, this vol., pp. 29–45.

THE BACTERICIDAL ACTION OF RADIUM EMANATION.*

By HELEN CHAMBERS and S. RUSS.

In view of the possibility of the successful treatment of malignant conditions by means of the rays from radium, an extended knowledge of their biological effects is desirable. The present investigation is concerned with the action of the various rays from radium upon the commoner pathogenic bacteria.

Experiments have been made by several observers† to find whether the rays emitted by radium have a bactericidal action. The general conclusion drawn from them is that these rays, especially those of the alpha and beta types, prevent the growth of certain bacteria. The bacteria under investigation in such experiments have been irradiated upon media in which they normally multiply. Although the general tendency of such observations rendered it improbable that the bactericidal action of the rays was really due to some effect upon the media, it seemed desirable to remove this objection, if possible. Experiments have accordingly been made by exposing suspensions of bacteria in distilled water to the various rays from radium. After irradiation, measured volumes of the bacterial suspension were planted on agar, and the number of colonies which developed compared with the number from an equal volume of the control suspension.

The organisms used in different series of experiments were *Staphylococcus pyogenes aureus*, *Bacillus coli communis*, *Bacillus pyocyaneus*, *Bacillus anthracis*, and *Bacillus tuberculosis*. The main conclusion to be drawn from these

* Read at the Laboratory Meeting of the Pathological Section of the Royal Society of Medicine held at the Cancer Research Laboratories, Middlesex Hospital, on April 2, 1912. Part published in "Proc. Roy. Soc. Med.," 1912, vol. v., p. 198.

† Strebel, "Fortschritte auf dem Gebiete der Rontgenstrahlen," iv., p. 125; Aschkinass and Caspari, "Pflugers Archiv. f. die ges. Phys.," Bonn, 1901, lxxxvi., pp. 603-18; Pfeiffer and Friedberger, "Berl. klin. Wochenschr.," 1903, xl., p. 641; Hoffmann, "Hygienische Rundschau," 1903, xiii. p. 914; Dixon and Wigram, "Dublin Journ. of Med. Sci.," 1904, cxvii., p. 161; Goldberg, E. S. London, "Das Radium in der Biologie und Medizin," Leipz. 1911, p. 28 *et seq.*

observations is that the alpha and beta rays from comparatively small quantities of radium—i.e., a few milligrammes—have a direct bactericidal action.

It has previously been shown* that the polymorphonuclear leucocytes of human blood suffer a reduction in their phagocytic power and are eventually destroyed when exposed to alpha rays, also that the opsonin in normal serum is destroyed by these rays. With a view to some possible clinical applications of the bactericidal effects, a series of observations has been carried out, with known quantities of radium emanation, upon the destruction of three of the elements that enter into the process of phagocytosis—namely, leucocytes, opsonin, and bacteria. The result of this investigation is that the destructive action of the rays upon the bacteria, in this case *Staphylococcus pyogenes aureus*, is much more marked than upon the two other constituents.

Methods of Experiments upon Bacteria.

The method by which the bactericidal action has been studied is as follows: The growth is removed from a twenty-four hours' agar culture and centrifugalised in 10 c.c. of sterile distilled water to wash the organisms. The fluid is pipetted off and 3 c.c. of fresh distilled water added to the bacterial deposit, which is then well shaken. About 2 c.c. of this emulsion are run into a small glass bulb (volume usually 10 c.c.) provided with two taps and containing a measured quantity of radium emanation; the remainder of the emulsion serves as the control. The two emulsions are then placed in the ice-chest at about 4° C.

The emanation is partially dissolved by the emulsion. The solubility coefficient for distilled water at 0° and 76 cm. pressure has been determined by Boyle† and found to be 0·51. Hence if the concentration of the emanation in the air within the bulb were 1 milli-curie per cubic centimetre‡ it would be about 0·5 milli-curie per cubic centimetre in the emulsion. The concentrations recorded in the paper refer to the number of milli-curies divided by the volume of the bulb. Throughout

* Chambers and Russ, "Proc. Roy. Soc.," Lond., 1911, lxxxiv., B, pp. 124–36.

† Boyle, "Phil. Mag.," December, 1911.

‡ One milli-curie is the amount of emanation in equilibrium with 1 mgrm. of pure radium.

the fluid the bacteria are subjected to the alpha, beta, and gamma rays from the emanation and its short-lived products, RaA, RaB, and RaC.

After any desired intervals a measured volume of the emulsion is withdrawn from the bulb and planted upon an agar slope, the same volume of the control being planted for comparison.

A series of tubes having been prepared in this manner for different times of exposure, they were incubated at 37° C.

Staphylococcus pyogenes aureus.—The bactericidal action of the emanation upon this organism may be seen from Fig. 1. The number of colonies gradually diminishes and eventually the fluid is sterile. The concentration of emanation in this series was 0·5 milli-curie per cubic centimetre. The control emulsion contained 3×10^9 organisms per cubic centimetre. It will be seen from the gradual diminution of growth that a completely lethal effect was obtained after five hours. As portions of the irradiated emulsion were removed from the bulb at various intervals for the above series, simultaneous observations were made upon stained films. The organisms

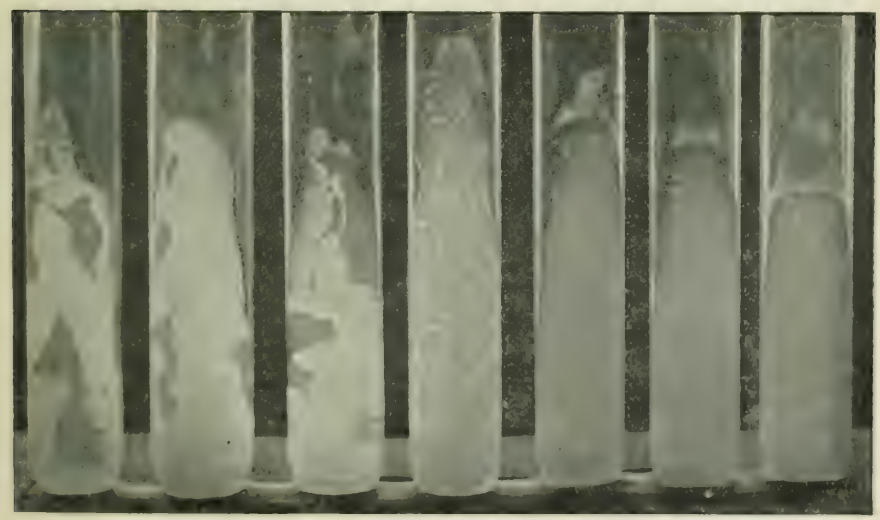


FIG. 1.

Action of radium emanation on *Staphylococcus pyogenes aureus.*

showed no difference in their staining power after irradiation. After several hours agglutination had occurred. With more prolonged exposure this effect was sufficiently marked to be seen by the naked eye. The emulsion, originally neutral to litmus, gradually became acid. When the exposure had lasted sufficiently long for a completely lethal action, the emulsion was removed from the bulb, and in order to see whether the fluid, acid in reaction, had any direct lethal action upon the organisms, it was centrifugalised and the clear supernatant fluid pipetted off. One volume of this fluid was added to an equal volume of the control emulsion and allowed to remain at room temperature. A control was provided by mixing one volume of the emulsion and one volume of distilled water. No agglutination was caused by the acid fluid. After twenty-four hours equal volumes from the two tubes were planted and incubated. The growth was in each case copious. After three days equal volumes were again planted and no difference in the two growths was detected. This indicates that the lethal action upon the organisms is a direct one, and is not to be attributed to changes in the fluid as a result of the irradiation.

Bacillus coli communis.—Emulsions of this organism in distilled water were exposed to the emanation, and the

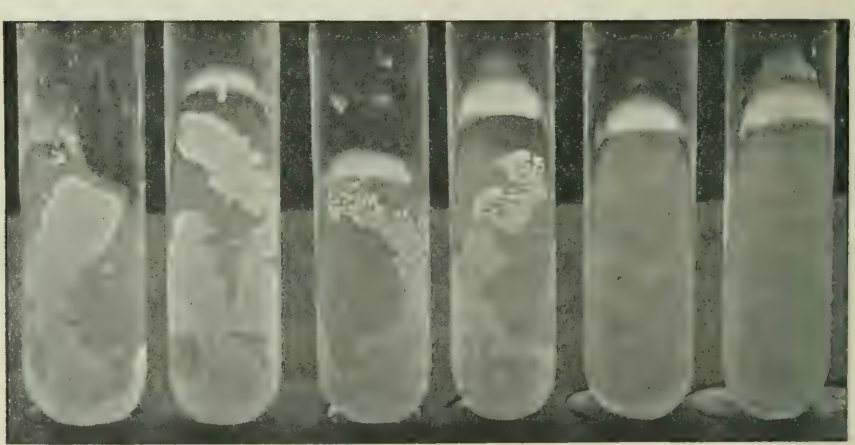
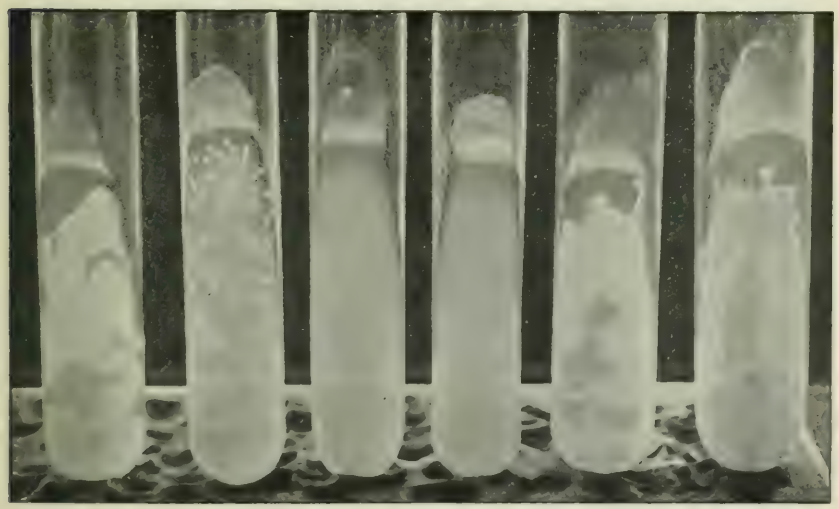


FIG. 2.

Action of radium emanation on *Bacillus coli communis*.

gradual diminution in the number of colonies for various times of exposure obtained in the manner indicated. Fig. 2 gives the result obtained with a concentration of 0·36 milli-curie per cubic centimetre, the emulsion containing $2{\cdot}5 \times 10^8$ bacteria per cubic centimetre. Fig. 3 exhibits the contrast in effect obtained with the emanation and X-rays. The same emulsion was used in the two cases; 0·67 milli-curie per cubic centimetre had a completely lethal action in four hours. Irradiation by soft X-rays for periods of three and six and a half hours had apparently a small inhibitory effect upon the number of colonies. Agglutination was observed after exposure to the emanation, and the emulsion, originally neutral to litmus, gradually became acid. A series of observations with the clear fluid after centrifugalisation of the emulsion similar to that detailed for *Staphylococcus pyogenes aureus* showed that the *Bacillus coli communis* was appreciably affected by the acidity of the solution. A part of the original emulsion, after being mixed with an equal volume of the acid fluid and allowed to stand for twenty-four hours, showed a diminution in the number of colonies compared with the



Control. 2 4 6 3 6½ hours.

Radium emanation. X-rays.

FIG. 3.

Action on *Bacillus coli communis*.

control, and this was more pronounced for longer intervals. The time taken, however, namely, several days, for a completely lethal action showed that the marked effects obtained with the emanation were not attributable to this indirect action.

Bacillus anthracis.—An emulsion in distilled water of anthrax bacilli from a twenty-four hours' agar culture was exposed to a concentration of 0·55 milli-curie per cubic centimetre. Equal volumes of the irradiated fluid were planted on agar after various times of exposure and incubated. From Fig. 4 it will be seen that an almost complete lethal effect was obtained after three hours. Marked agglutination was observed after one hour's exposure. This is shown by the photomicrographs in Fig. 5, which represent films of the control and irradiated emulsions one hour after exposure had begun. After twenty-four hours' exposure the emulsion was centrifugalised and the effect of the supernatant fluid on an equal volume of the control emulsion tested. As in the case of *Staphylococcus pyogenes aureus*, the supernatant fluid

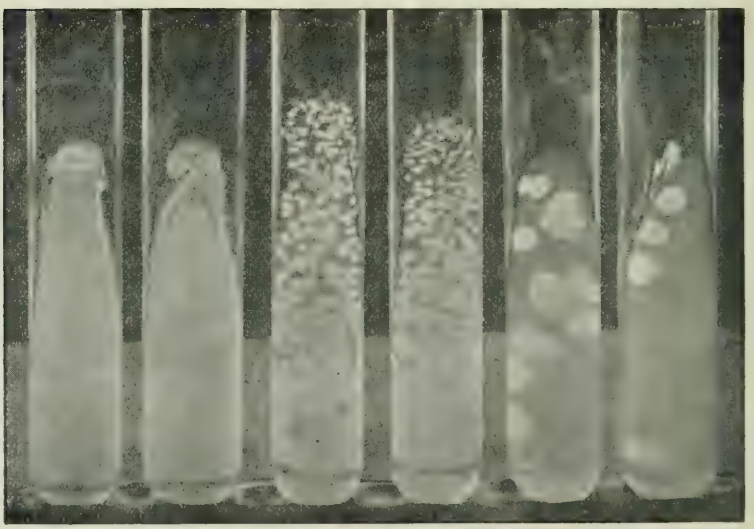
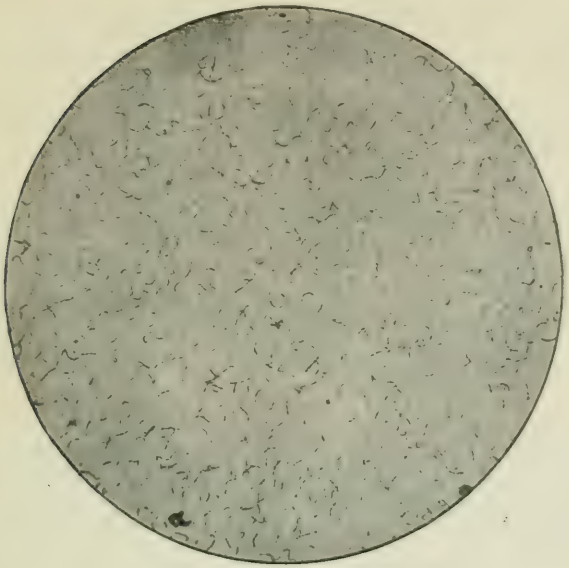
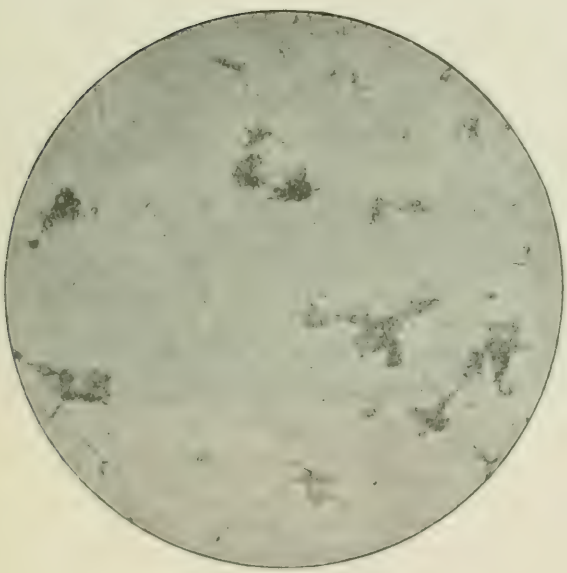


FIG. 4.

Action of radium emanation on anthrax bacilli.



Control.



Experimental.

FIG 5.

Agglutination of *Bacillus anthracis* by radium emanation.

had practically no lethal action upon the subsequent growth of the anthrax bacilli, and did not cause agglutination.

Anthrax Spores.—An emulsion of anthrax spores was found to be more resistant to the action of the emanation than the organisms hitherto dealt with. The result of exposing a thick emulsion, previously heated to 80° C. for half an hour, to 0·81 milli-curie per cubic centimetre may be seen from Fig. 6. After an exposure of six hours to a more intense radiation than was used in any of the previous cases a small growth was still obtained. After more prolonged exposure, however, the spores failed to grow at all.

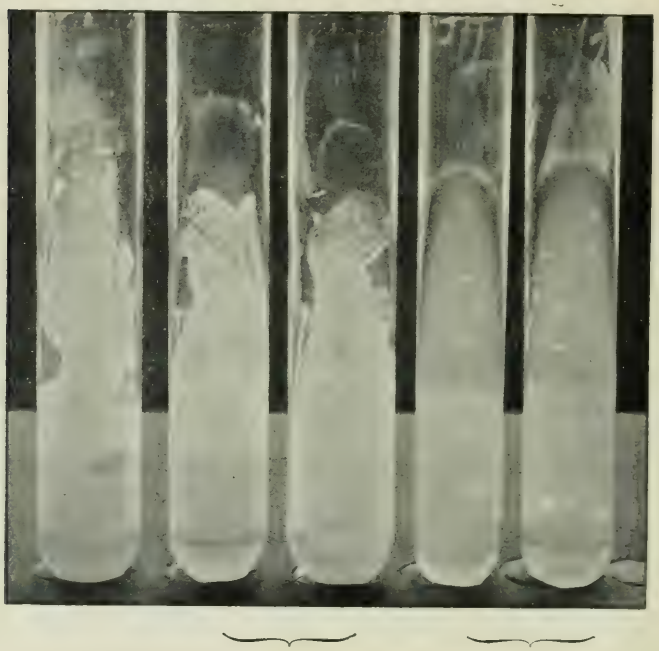


FIG. 6.

Action of radium emanation on anthrax spores.

Agglutination was observed after six hours, and the fluid had become acid to litmus. The pathogenicity of the organism

was shown by the subcutaneous inoculation of a mouse. The animal died in twenty hours, and anthrax bacilli were cultivated from its blood.

Tubercle Bacilli.—A sample of sputum containing numerous tubercle bacilli was exposed to ·30 milli-curie per cubic centimetre. During the irradiation 1 cubic centimetre was removed and ·5 cubic centimetre injected into each of two guinea-pigs; this was done for four different time intervals, namely, $1\frac{1}{2}$, $3\frac{1}{2}$, $5\frac{1}{2}$, and 8 hours. Two animals were inoculated with the same volume of the untreated sputum to serve as controls.

The animals were for the most part kept under observation for six weeks; they were then killed and post-mortem examinations made.

The result of this experimental series is to show that prolonged exposure to the emanation is lethal to tubercle bacilli, as may be seen from the results in Table I.

TABLE I.

Time of Irradiation.	Result of Post-mortem Examination.
0	(1) After four and a half weeks the animal showed extensive tuberculous disease, with caseous inguinal glands and numerous miliary tubercles in the liver and spleen. Tubercle bacilli were found in stained films.
	(2) After 5 weeks the animal showed the same as 1.
$1\frac{1}{2}$ hours ...	(3) After four and a half weeks the right inguinal, iliac, and lumbar glands were enlarged and caseous, the liver and spleen containing numerous miliary tubercles.
	(4) After five weeks the animal died, the post-mortem findings being practically identical with (3).
$3\frac{1}{2}$ hours ...	(5) After six weeks the right inguinal, iliac, and lumber glands were caseous.
	(6) After six weeks no tuberculous disease was found.
$5\frac{1}{2}$ hours ...	(7) The animal died after nine days. A few isolated tubercle bacilli were found in sections of the subcutaneous tissue at the site of inoculation.
	(8) After six weeks no tuberculous disease was found.
8 hours ...	(9) " " " "
	(10) " " " "

The caseous material of the glands from animal (1), which contained numerous tubercle bacilli, was emulsified with about twice its volume of normal saline and exposed to ·42 milli-curie per cubic centimetre. After periods of irradiation of 45 min., 1 h. 40 m., 3 h. 10 m., and 5 hours, ·5 c.c. was removed and ·25 c.c. injected into each of two guinea-pigs. The animals were killed after six weeks and post-mortem examinations made.

Owing to the increased quantity of emanation, and probably also to the greater facility of its diffusion through the tuberculous fluid than in the previous case, a completely lethal action was obtained in a shorter time, namely, 1 h. 40m., as may be seen from the data in Table II.

TABLE II.

Time of Irradiation.	Result of Post-mortem Examination.
0 h. 45 m. ...	(1) After six weeks the right inguinal and lumbar glands were caseous, and the liver and spleen contained numerous miliary tubercles.
	(2) ,, ,, ,, ,,
1 h. 40 m. ...	(3) After six weeks no tuberculous disease found.
	(4) ,, ,, ,,
3 h. 10 m. ...	(5) ,, ,, ,,
	(6) ,, ,, ,,
5 h. 0 m. ...	(7) ,, ,, ,,
	(8) After eight days the animal died.

From the results of this series it might reasonably be hoped that an injection of salt solution containing the emanation in a concentration of about 1 milli-curie per cubic centimetre into a tuberculous focus might remain in the vicinity sufficiently long for a lethal effect upon the organisms.*

* A convenient arrangement for this purpose is a cylinder one end of which is closed by a rubber cap, the other by a tap through which the emanation enters. When required a hypodermic syringe is filled by inserting the needle through the rubber cap and drawing off the required volume of saline.

The Effect of Radium Emanation upon Motile Bacilli.

The bactericidal action of the emanation upon *Bacillus pyocyaneus* is well marked, and its gradual destructive effect has been observed with the aid of the simple piece of apparatus of Fig 7, which allows of continuous observations being made upon the motile bacilli while under the influence of the emanation. It consists of a shallow brass box provided with side tubes for the in- and out-flow of the gas, and two glass windows in the base to allow of microscopic observations of hanging drops of the bacilli when placed over the apertures A and A^1. The hanging drops having been set up on slips of mica, the air is displaced by the emanation and continuous observations begin.

With a concentration of about 0·5 milli-curie per cubic centimetre in the box (volume about 5 c.c.) a diminution in

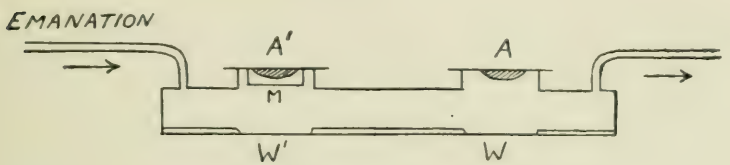


FIG. 7.

motility is noted in one hour; thence onward there is a tendency to agglutinate, and ultimately no motion is observed beyond Brownian movement. A platinum loop culture of the organisms at this stage fails to give any growth; in fact, before this stage is reached, when a movement of the bacilli is still visible, no growth is found to follow inoculation on agar. This is probably because the emanation goes into solution in the drop, and its effects continue even after removal from the field of observation.

To show the special effectiveness of the emanation when dissolved in the bacterial emulsion, experiments were carried out in the following way. A film containing a hanging drop was placed over the aperture A, so that the emanation was free to go into solution in the drop; over the other aperture, A^1, was placed another hanging drop protected by a very

thin sheet of mica which prevented the direct access of the emanation to the drop, but effected practically no absorpti n of the α and β rays from the emanation within the box. The organisms in the uncovered drop quickly succumbed to the effect of the emanation, whereas in the other they were still moderately motile after an exposure of several hours. As a typical example may be cited the exposure of two such drops to a concentration of 0·52 milli-curie per cubic centimetre. After three hours' exposure platinum loop cultures were made from the two drops. That from the uncovered drop remained sterile, whereas a moderate growth was obtained from the covered drop.

Quantitative Destruction of the Elements concerned with Phagocytosis.

The rays emitted by the emanation and its products are, generally speaking, destructive in their action. In view of some possible clinical applications of the bactericidal action of the emanation, it was desirable to make a quantitative estimation of its effects upon three of the constituents that enter into the process of phagocytosis—namely, bacteria, leucocytes, and the opsonin contained in normal serum. The result of this investigation is to show that the bacteria are much more affected by the emanation than the two other constituents.

(1) The procedure already described for measuring the bactericidal action was extended so as to obtain counts of the relative number of living bacteria in portions of the emulsion after various times of exposure. Measured volumes were diluted in sterile distilled water in steps of one hundred or less as required. Agar tubes were inoculated with equal volumes of these dilutions and plate cultures made. After incubation, counts were made of the relative number of colonies occurring in the various dilutions. The data contained in Table III. refer to an emulsion of *Staphylococcus pyogenes aureus* in distilled water containing 3×10^9 organisms per cubic centimetre exposed to 0·48 milli-curie per cubic centimetre at about 4° C.

TABLE III.

Exposure to emanation, time in minutes.	Number of colonies in 3×10^{-2} c.mm.	Logarithms of numbers.
5	3,040	3·483
10	1,090	3·037
15	300	2·477
20	190	2·279
30	32	1·505
40	3	0·477

From the numbers in column 2 and the graph in Fig. 8 it will be seen that after an exposure of half an hour to the emanation the number of living bacteria is reduced to 1 per cent. of those initially present. If the logarithms of the numbers be plotted against time the points do not depart from a straight line more than can be attributed to experimental errors. This indicates that the destruction of the bacteria occurs at an approximately exponential rate.

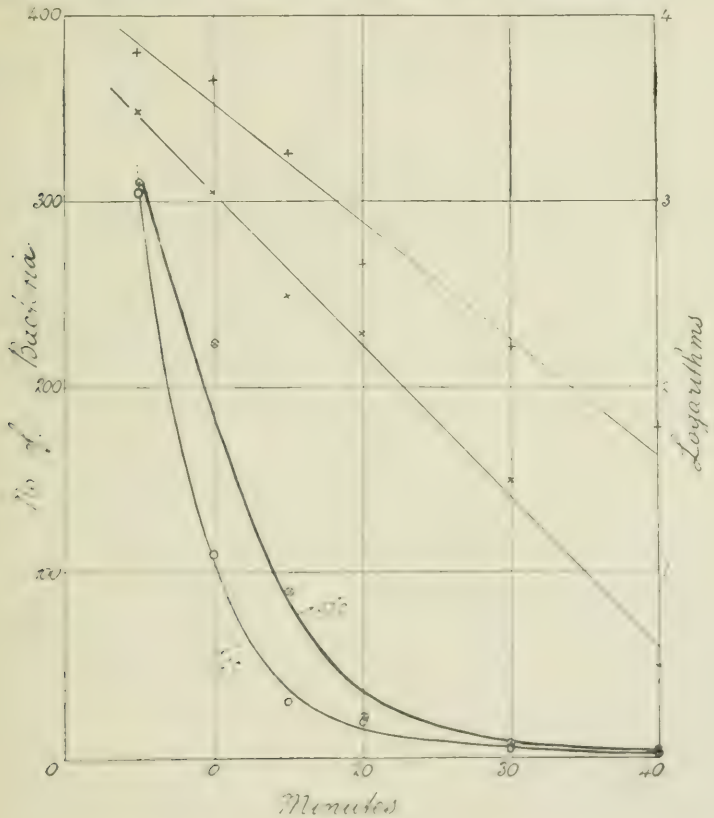


FIG. 8.

Under the conditions of this experiment, namely, at 4° C., the bacteria do not multiply. To see whether the bactericidal action of the emanation was in any way altered when the bacteria were placed under conditions favourable to their proliferation, an emulsion of *Staphylococcus pyogenes aureus* in broth at 37° containing 2×10^9 organisms per cubic centimetre was exposed to 0·2 milli-curie per cubic centimetre, and a series of observations made after various times of exposure in the manner detailed above. The number of colonies obtained from the same volume of irradiated fluid gradually diminished with time, as may be seen from the data in Table IV. and Fig. 8.

TABLE IV.

Exposure to emanation, time in minutes.	Number of colonies, in 10^{-2} c.mm.	Logarithms of numbers.
5	6,200	3·792
10	4,450	3·648
15	1,800	3·255
20	445	2·648
30	162	2·210
40	60	1·778

By plotting the logarithms of the number of colonies against time, it was found that the destruction proceeds at an approximately exponential rate. From the slopes of the two logarithmic lines at 4° and 37° it was found that the rate of destruction was 1·32 times as great in the former as in the latter case, with, however, an intensity of emanation 2·4 times that which was experienced at 37° C. It seems, therefore, that the rate of destruction of bacteria by the emanation is not hindered under conditions favourable to their growth.

(2) The gradual diminution in phagocytic power of human leucocytes when exposed to the emanation was found by exposing them, after being washed in normal saline, to 1·6 milli-curies per cubic centimetre at 4° C., and after various periods of irradiation their phagocytic power was compared with that of a control portion of the same suspension of leucocytes. No degeneration was observed in the leucocytes during a period of eight hours' irradiation. Subsequently degenerative changes, vacuolation of the protoplasm, and defective staining of the nucleus, became increasingly evident, until eventually the leucocytes were destroyed. The procedure was that usually adopted for opsonic determinations,

two volumes of the leucocyte emulsion being added to one volume of an emulsion of *Staphylococcus pyogenes aureus* and one volume of normal serum. The data contained in Table V. and the graph in Fig. 9 show the gradual reduction in phagocytic power of leucocytes when irradiated at 4° C.

(3) The quantitative reduction in opsonin, *as evidenced by the usual opsonic estimations*, under the action of the emanation was obtained by exposing normal serum to 1 millicurie per cubic centimetre at 4° C. After various periods of irradiation the opsonic content of the serum was compared with that of the control portion by estimating the phagocytosis of an emulsion of washed leucocytes when mixed with an emulsion of *Staphylococcus pyogenes aureus* and either of the two sera. The gradual diminution in opsonin when the serum is irradiated is shown in Table VI. and Fig. 9

TABLE V.

Exposure to emanation, time in hours.			Number of organisms in 200 leucocytes.			Percentage of the control phagocytosis.
Hours.	Minutes.		Experimental.	Control.		
3	0	...	1,200	1,290	...	93
4	45	...	880	1,136	...	77
7	5	...	648	808*	...	61
				(Corrected 1,060)		
8	0	...	468	1,008	...	46
9	50	...	464†	780	...	—

TABLE VI.

Exposure to emanation, time in hours.			Number of organisms in 200 leucocytes.			Percentage of opsonin.
Hours.	Minutes.		Experimental.	Control.		
1	30	...	520	622	...	77
3	30	...	267	605	...	44
5	30	...	275	596	...	46
7	15	...	223	636	...	35
9	15	...	143	558	...	26

Spontaneous phagocytosis was found to be 8 per cent. of the mean of the controls. It was thought unnecessary to correct the curve on this account. The behaviour of the three quantities under consideration upon exposure to the emanation is represented graphically in Fig. 9. The intensity of the emanation in this series was 1·6, 1, and 0·48 milli-curies per cubic centimetre for the leucocytes, opsonin, and bacteria

* The third control was subject to a technical error and is corrected to the value indicated by the remaining four observations.

† Numbers of degenerate leucocytes were observed, but not included in this count. Consequently the percentage of the control phagocytosis is not available.

respectively. They are quantitatively affected in the inverse order: whereas the colonies grown from the bacteria were reduced to 1 per cent. of the number obtained from the control after an exposure of half an hour, the leucocytes

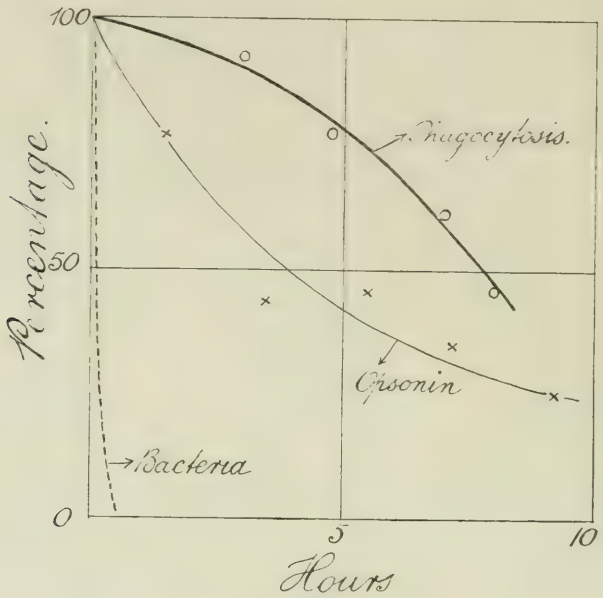


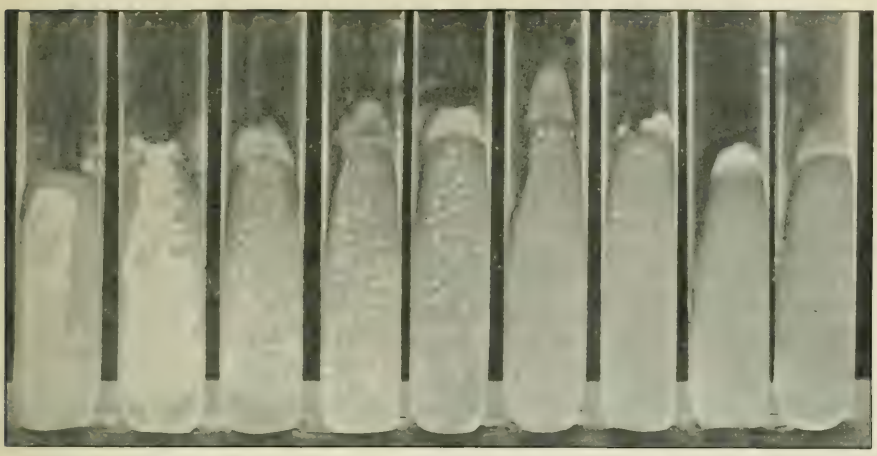
FIG. 9.

when exposed to three times the intensity of emanation only suffered a reduction of 50 per cent. in their phagocytic power after about seven hours' irradiation, and the opsonin, on exposure to twice the intensity of emanation, was reduced to 50 per cent. in about four hours.

The Action due to the α and β Rays.

Emulsions irradiated in the manner described were exposed to the α, β, and γ rays from the emanation and its products. The bactericidal effects which have been observed are due to the α and β rays. Exposure of an emulsion of *Staphylococcus pyogenes aureus* to the γ rays only, from 7 mgr. of radium bromide, gave no evidence of any effect after an exposure of one week. When the β rays from this source were utilised a

completely lethal effect was obtained in six hours, as may be seen from the series (Fig 10).



Control. 2 3½ 4 4½ 5 5½ 6 7¾ hours' exposure.

FIG. 10.

Action of β rays on *Staphylococcus pyogenes aureus*.

CONCLUSIONS.

1. Radium emanation in concentrations of less than a millicurie per cubic centimetre has a marked bactericidal action.

2. Agglutination of bacteria in distilled water is an early sequel to their irradiation.

3. Bacteria are more quickly destroyed by the emanation than are opsonin and leucocytes.

NOTE ON THE AMBOCEPTOR COMPLEMENT HÆMOLYTIC REACTION.

By HELEN CHAMBERS and S. RUSS.

There are two prevalent explanations of the hæmolytic reaction that takes place when complement is added to red blood corpuscles which have been sensitised by a suitable amboceptor. These explanations are based upon chemical or upon adsorption considerations. On either view the stroma of the red corpuscle is held to be the seat of the reaction. If the process be of an essentially chemical nature, it is of importance to decide whether the hæmolytic reaction proceeds in an analogous manner to mono- or bi-molecular reactions.

When a mixture is made of fully sensitised red-blood corpuscles and complement and kept at 37° C. hæmolysis results. A series of measurements of the amount of hæmoglobin liberated at various times showed that the quantity Q_t obtained at any time t may be expressed in terms of the final value Q by the equation

$$Q_t = Q\,(1 - e^{-\lambda t}) \qquad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where $\lambda = \cdot 25$, the time being measured in minutes; this is represented by the curve in Fig. 1.

A case of this kind is cited by Arrhenius* (being an observation by Henri of a similar nature to that above) as following very closely the law of mono-molecular reactions. It seems, however, that the amount of free hæmoglobin is not only a measure of the extent of the amboceptor-complement reaction, but also of the gradual diffusion of the hæmoglobin out of the blood corpuscles.

The rate of the hæmolytic reaction is markedly dependent upon temperature. If the mixture of the components be made

* Arrhenius, "Immuno-Chemistry," p. 106.

at 27·8° and 17° C. the diminished rates may be seen from the two other curves, Fig. 1. The times in minutes refer to the experiments at 37° and 27·8° C., and in hours to the observation at 17° C. No hæmolysis results even after several days if the mixture is kept at 0° C.

These observations were all made with a mixture of one volume of fresh human serum and four volumes of a 20 per cent. suspension of sheep's corpuscles containing ten hæmolytic doses of amboceptor, suitable controls being retained.

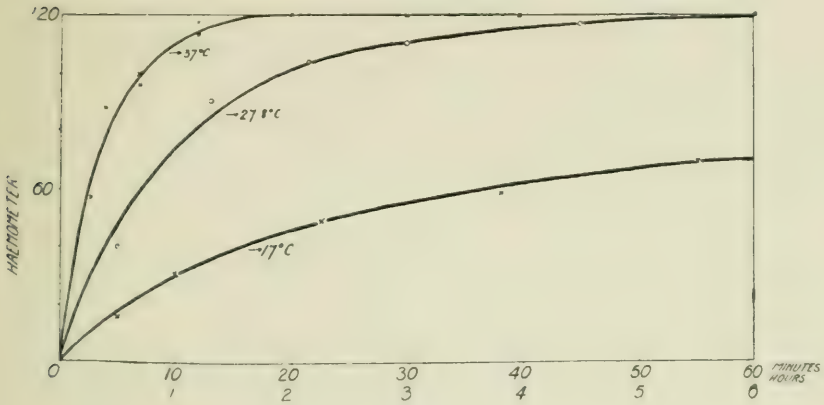


FIG. 1.

The mixture was distributed equally among a series of thin test tubes which were placed in a water bath so that the temperatures were quickly attained. This was tested by means of a thermo-electric junction placed along the axis of the tube containing the corpuscle suspension. When placed in a water bath at 37° C., the thermo-electric junction attained a temperature of 34° C. in 30 seconds and 36° C. in 42 seconds. The estimation of the amount of free hæmoglobin in a measured volume of the clear fluid pipetted off after centrifugalisation was made with a Sahli hæmometer.

If a series of tubes containing the hæmolytic mixture be placed in a water bath at 37° C., and after a certain interval of time they are removed and placed in water at 0° C., the amount of free hæmoglobin gradually increases, although the

initial part of the reaction (chemical or adsorptive in nature) does not take place at all at this temperature. Curves I. and II. in Fig. 2 correspond to cases in which this reaction was allowed to occur at 37° C. for 1 and 2 minutes respectively,

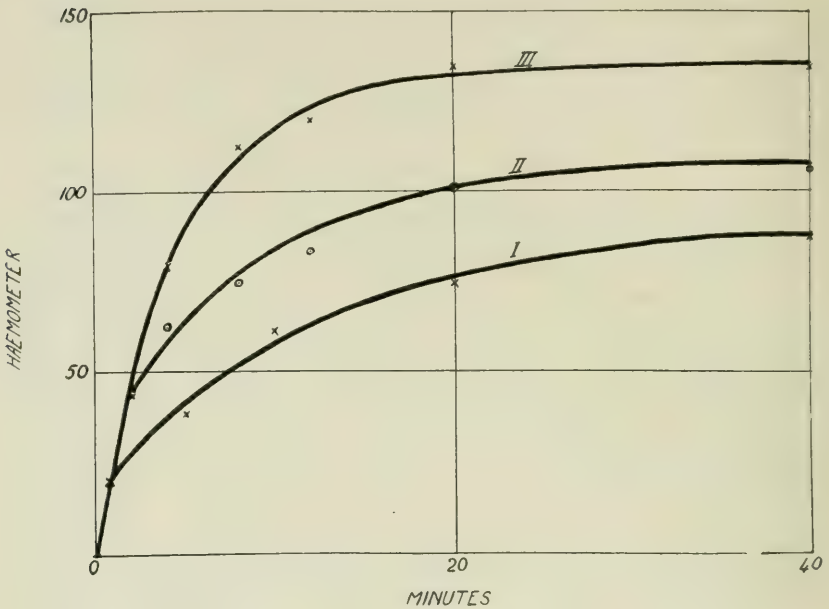


Fig. 2.

after which the diffusion of hæmoglobin from the corpuscles occurred at 0° C. From Curve III. in the same figure, which illustrates the continuous progress of the reaction at 37° C., it may be seen that after 1 minute 18 per cent. of the final amount of hæmoglobin was obtained, and after 2 minutes 33 per cent. Cutting off the initial part of the reaction at these times, and allowing diffusion alone to take place for 40 minutes at 0° C., 64 per cent. and 78 per cent. of the same final value were obtained in these two cases.

Further observations on these lines showed that the initial part of the reaction (the interaction of the complement and amboceptor on the stroma of the corpuscles) is complete in less than 5 minutes at 37° C., but that, owing to the time necessary for the hæmoglobin to diffuse out of the corpuscle, the process is apparently traceable for quite

20 minutes (*vide* Curve III.). It seems, therefore, that this curve actually represents two distinct processes: one the reaction, chemical or adsorptive in nature; and the other the diffusion of the hæmoglobin out of the corpuscles.

It is not a little remarkable that, under these circumstances, a curve approaching so closely to the simple exponential type should be found to represent the quantity of hæmoglobin released at various times. This is probably to be explained by the marked difference in the rates of the two processes involved. At lower temperatures, as the rate of the reaction approaches that of the diffusion process, the agreement with a simple exponential expression is not so close.

The rate of the whole process may be characterised by equation (1), the value of λ being ·25 at 37° C. and ·05 at 0° C., when allowance is made in the latter case for the hæmoglobin liberated during the time that the corpuscles were at 37° C. The reduction in the value of λ at 0° C. to one-fifth of its value at 37° C. shows the dependence of the rate of the diffusion process upon temperature, and may be contrasted with that of the amboceptor-complement reaction proper. From Fig. 1 it is seen that equal hæmometer readings are obtained at the temperatures 37°, 27·8°, and 17° C. at times 3·3 minutes, 9 minutes, and 5½ hours respectively; the rates, therefore, being in the ratio of 100 : 36·6 : 1. The temperature coefficient of the reaction calculable from these ratios is probably a maximum owing to the diminished rate of diffusion at the lower temperatures. It is not possible, however, from the observations to make an accurate correction allowing for this.

These experiments, while not disproving the chemical nature of the reaction in question, show that an exponential rate of release of hæmoglobin is not, in this case, evidence of a mono-molecular reaction.

THE EFFECT OF RONTGEN AND RADIUM RADIATIONS UPON THE VITALITY OF THE CELLS OF MOUSE CARCINOMA.

By B. H. WEDD and S. RUSS.

(*Communicated to the Pathological Society of Great Britain and Ireland on January 5th*, 1912.)

It has been shown by several observers that an undoubted effect is produced upon mouse tumours *in vivo*, by the action of X-rays and radium radiations.

Apolant (1904) (1), among others, produced a rapid absorption of these tumours by the application of radium. Histologically a proliferation of the connective-tissue stroma is the most marked feature of tumours undergoing absorption by this treatment. This result has been attributed to a direct selective action upon the tumour cells, and to a secondary effect produced by the reaction of the surrounding tissues. Werner (1904) (11) obtained evidence that the products of the decomposition of lecithin under the influence of radium, which had been demonstrated by Schwartz (1903) (9) and Schafer (1904) (8), could produce effects upon normal tissues similar to those produced by radium itself. Bashford (1905) (2) attributed the beneficial effect of radium to the proliferation of the stroma; he regarded the destruction of the tumour cells as a secondary effect, and produced evidence that this proliferation is associated with hæmorrhages from the delicate capillaries of the stroma.

In experiments performed upon tumour tissue *in vivo* it is difficult to exclude the effects produced by the reaction of

the tissues of the animal. The experiments to be described have been performed under conditions which exclude this factor.

Jensen (1904) (7) demonstrated the destructive effects of certain agents upon excised tumours; these included heat, Finsen light, and carbolic acid; Shattock and Dudgeon (1910) (10) showed that tumour tissue, if completely dried, cannot proliferate when inoculated. Clunet (1910) (5) submitted tumour tissue to X-rays without demonstrating any destructive action, but his experiments seem to have been confined to rays filtered through the cover of a Petri dish.

In the following experiments tumour tissue freshly excised under aseptic conditions has, subsequent to irradiation, been transplanted into other mice, and its power of proliferation compared with control portions of the same tumour.

In general it has been found that excised tumours sufficiently irradiated by X-rays or the β-rays from radium lose their power of proliferation on transplantation. Soft X-rays are more effective than those of a more penetrating type.

The tumour used for these experiments was one for which we are indebted to Dr. Bashford. Histologically it is an adeno-carcinoma of the mamma of the mouse. When first received it showed a mixed acinous and alveolar structure, but is now almost entirely of the alveolar type. The appearance of the tumour in section can be seen in Fig. 8, Plate IV., which is a microphotograph of a tumour eight days after inoculation.

This tumour gives a high percentage of successful results on inoculation and forms large tumours before degeneration is advanced; these features rendered it suitable for the experiments to be described. Its slow growth, which necessitated a long interval before a positive result could be recorded without killing the animal, and its occasional tendency to spontaneous absorption were less favourable features. The occurrence of secondary deposits in the lung was detected microscopically in one case, but macroscopic evidence of malignancy other than those of direct extension and progressive growth has not been noted.

Experimental Arrangements.

Irradiation by X-rays.

The general method of procedure when irradiating the tumours with X-rays is indicated by Fig 1. Thin slices, about 2 mm. thick, of a tumour from a freshly-killed mouse were moistened with normal saline and placed in a watch-glass (*W*), and covered with a thin sheet of mica (*M*) (0·01 mm. thick). The watch-glass was placed inside a Petri dish and surrounded by water for cooling purposes. This system was placed about 2 cm. below an X-ray bulb and an exposure given for any desired interval. The tumour was then removed and transplanted in small sections into a number of mice at the axilla; part of the original tumour was retained as control and transplanted into a comparable number of mice.

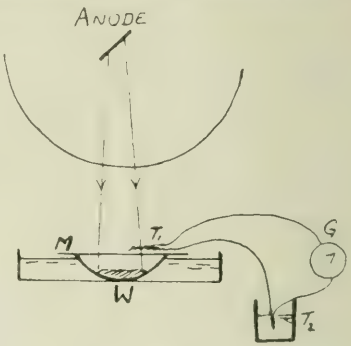


Fig. 1.

For the earlier experiments the irradiated and control portions of the tumour were inoculated into different mice. After the experiment had been tried of planting the control tumour upon the opposite side to the irradiated in the same mouse with satisfactory results, this procedure was usually adopted. The results obtained by the two methods may be seen in Table I., in which are collected the totals of mice inoculated with control tumour and the percentages of successful growths obtained. These results show that the method of planting the irradiated and control tumour in the same mouse does not appreciably affect the result. Not only is this an economic measure, but any error arising from variation of susceptibility in the different mice is thus obviated. In a few cases when two portions of the same tumour were inoculated into the same mouse, one portion developed while the other did not, thus showing that other factors besides susceptibility, probably connected with the fragment of tumour selected or with manipulations employed, determine the subsequent growth of the tumour; this has previously been demonstrated by Bashford (1905) (3).

TABLE I.

	Mice.		Percentage of Growths.
	Inoculated.	Survived.	
Control and experimental tumours inoculated in same mice	125	88	62 per cent.
Controls in different mice	121	86	70 „

During the course of these experiments mice of various species and ages were used for the inoculations.

The mice were kept under similar conditions, and periodically examined for the growth of the tumours over a period of at least one month from the time of inoculation.

An X-ray bulb, especially when emitting a soft type of radiation, warms appreciably when worked for long periods. The temperature of the water in the Petri dish in most of the experiments was read by a thermometer and never exceeded 25·6° C. In the later observations a copper-nickel thermo-electric junction was used. One junction (T_1) was placed on the mica sheet (M), the other in water at room temperature, the readings of a distant dead-beat galvanometer (G) allowing the temperature to be read more accurately and conveniently. That the slight increase in temperature, usually about 5° C., did not account for the inhibited growth of the tumour was proved by warming part of a tumour to 28° C. for five hours and transplanting it. The tumour grew equally well with the control portion kept at room temperature.

Initial experiments were made by exposing excised tumours for three hours to composite X-rays. On three separate occasions such tumours showed no signs of proliferation when transplanted.

A series of observations was then made by varying the time of exposure between five minutes and two hours.

The character of the X-rays for this series was of a moderately soft type, the spark-gap ranging from 6 to 10 cm.

From Table II. it is seen that an exposure of half an hour has an appreciable inhibitory effect upon the proliferating power of the tumour. No growth of the tumour has been observed subsequent to irradiation lasting two hours by unscreened X-rays of a moderately soft type.

Out of the seventy controls which survived, 67 per cent. developed tumours.

TABLE II.

Time of Exposure	Number of Mice		Growth of Tumour.	
	Injected.	Survived.	Positive.	Negative.
0 hours 5 minutes	6	5	3	2
0 „ 15 „	11	9	4	5
0 „ 30 „	25	21	4	17
1 hour 0 „	17	13	1	12
1 „ 45 „	6	6	0	6
2 hours 0 „	30	25	0	25
Controls for the entire series ...	82	70	47	23

To determine the type of X-rays responsible for the marked inhibitory action, a series of exposures was undertaken in which the rays were varied from very soft to rays of considerable hardness, by working the coil at a spark-gap varying from 6 cm. to 16 cm. Owing to the fact that even when the coil was running at the longest spark-gap a certain number of soft rays was still present (as measured electroscopically), sheets of aluminium were interposed when it was desired to eliminate this soft type. The results may be seen in Table III.

TABLE III.

	Conditions of Radiation.					Number of Mice.		Growth.	
	Current.		Spark-gap.	Screen.	Time of Irradiation.	Inoculated.	Survived.	Positive.	Negative.
	Primary.	Secondary.							
	Amperes.	Milli-amperes.	Cm.						
Series 1.	4	0·8	6	None	15 minutes	5	4	1	3
	4	0·8	6	„	30 „	5	4	0	4
	4	0·6	8	„	1 hour	8	7	0	7
					Controls	13	11	10	1
Series 2.	4	0·7	6–7·5	0·56 mm. Al	30 minutes	8	4	3	1
	4	0·4		„	1 hour	8	5	0	5
	4	—	„	„	2½ hours	6	4	0	4
			„		Controls	21	13	11	2
Series 3.	4	0·4		1·12 mm. Al	1 hour	8	5	2	3
	4	0·4	14	„	1½ hours	8	8	6	2
	4	0·35	14	„	2 „	7	6	0	6
			16		Controls	23	16	9	7

Series 1 contains the results of a typical exposure to unscreened rays of a very soft type. A marked inhibitory action is observed after irradiation for half an hour.

The results in Series 2 were obtained with rays of a soft type. The tumour was, however, shielded from the very soft rays by a screen of aluminium 0·56 mm. thick. The elimination of this soft type of radiation necessitates an extension of the time of irradiation to prevent the growth of the tumour. The special effectiveness of the soft rays is brought out still more clearly in Series 3. In this case a bulb giving a penetrating type of rays was used, and the soft constituents of the beam eliminated by aluminium 1·12 mm. thick. The period now required to completely prevent the growth of the tumour was two hours.

It will be seen from Table III. that during the whole of these exposures the current through the primary was kept constant, the current through the bulb diminishing as the hardness of the rays increased.

Secondary Radiation.

The foregoing results having established that soft X-rays are particularly effective in preventing the growth of the tumour, it was decided to see whether such inhibitory action could be obtained by some soft type of homogeneous secondary radiation. The secondary radiation from copper was selected, as it is of a soft type, and is completely absorbed in going through a layer of tissue 2 mm. thick. The intensity of the secondary radiation from copper has not yet been accurately measured;* long exposures were, however, given under the following experimental conditions.

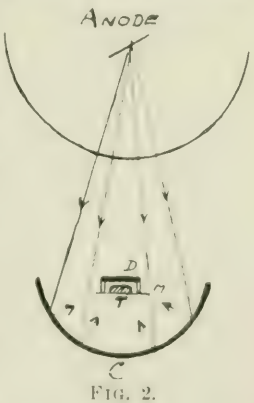


FIG. 2.

A thin slice of freshly excised tumour (*T*) was placed on a very thin sheet of mica (*M*) (0·01 mm. thick), shielded from the direct rays of the bulb by a small lead disc (*D*) (1·65 mm. thick and 1·5 cm. diameter), and

* Barkla states that under favourable conditions an intensity of 10 per cent. of that of the primary beam may be obtained.

mounted at the centre of a hemispherical copper bowl (*C*) (diameter 7·2 cm.). The direct rays meeting the copper caused it to emit homogeneous secondary rays, some of which radiated the tumour through *M*.

Two separate exposures yielded no evidence of any inhibitory action due to these secondary rays, as will be seen from Table IV.

TABLE IV.—SECONDARY RAYS FROM COPPER.

Time of Radiation.		Inoculated.	Survived.	Positive.	Negative.
2½ hours ...	Experimental	8	5	3	2
	Control	8	5	4	1
4 „ ...	Experimental	5	3	3	0
	Control	5	4	4	0

The negative result is probably due to the small intensity of the secondary rays compared with the primary. An approximate estimate of these intensities was obtained in the following way: A Sabouraud pastille was placed in the position previously occupied by the tumour, and after two hours' exposure to the secondary radiation, the change in tint of the pastille was the same as that produced by five minutes' exposure to the direct rays. It thus appears that prolonged exposures would be required for these secondary rays to exert any appreciable effect upon the tumour.

Irradiation of Mouse Tumours *in vivo*.

Two experiments were performed by inoculating a tumour which had been irradiated *in vivo*. The results obtained are interesting, but, owing to the enforced absence of controls, cannot be regarded as conclusive. In the first case, a rather small tumour was selected, irradiated for a period of four and a half hours, extending over three days, and the tumour excised and inoculated with the following results:—

	Mice Inoculated.	Survived.	Positive.	Negative.
10 days after inoculation ...	7	7	4	3
25 „ „ „ ...		7	3	4
39 „ „ „ ...		6		5

In contrast to the gradual development usual with this tumour, it will be seen that what was assumed to be an early proliferation of the tumour was subsequently followed by absorption.

In the second experiment, a mouse with a large tumour was irradiated during one day for a total period of three hours. The tumour was found to be considerably degenerated, and inoculation into sixteen mice, with pieces taken both from the superficial and deep parts of the tumour, was in no case followed by growth.

Irradiation by Radium Rays.

The following disposition of apparatus permitted the exposure of freshly excised tumours to the α, β, and γ rays of radium.

A thin slice of tumour (T) (Fig. 3) about 2 mm. thick was placed between two mica sheets ($M\,M$), the thickness of which was varied to allow or prevent the penetration of the α-particles through them.

The tumour was moistened with normal saline and enclosed by a vaseline ring ($V\ V$), which prevented evaporation. This system was placed between two radium capsules containing 3·3 and 2·3 mgrm. radium bromide respectively, thus being irradiated from above and below. Subsequent to irradiation for any desired interval, a similar procedure in the transplantation of the tumour to that which has been described was followed.

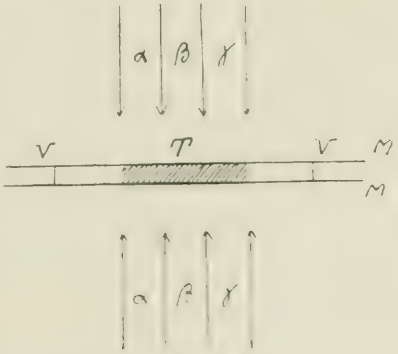


Fig. 3.

An initial exposure, lasting four hours, to the composite radium radiation prevented the subsequent growth of the tumour when transplanted into five mice, a control portion of the original tumour taking, in all the inoculations.

The small penetrating power of the α-rays (less than

0·1 mm. depth of the tumour) rendered it unlikely that they accounted for this effect. When these rays were eliminated by a screen, an exposure of half an hour was as effective in inhibiting growth as when the α-rays were included in the radiation, as may be seen from Table V.

By interposing lead screens 2 mm. thick between the radium and the tumour the β-rays were completely eliminated,

TABLE V.

Character of Rays.	Time of Irradiation.	No. of Mice.		Growth of Tumour.	
		Inoculated.	Survived.	Positive.	Negative.
α-, β-, and γ-rays	15 minutes	6	4	2	2
	30 „	6	4	3	1
	4 hours	5	3	0	3
β- and γ-rays...	30 minutes	14	10	6	4
	1 hour 30 minutes	8	4	0	4
	2 hours	6	4	0	4
	4 „	12	9	0	9
	Controls	37	29	18	11
γ-rays	$3\frac{1}{2}$ hours	8	7	3	4
	Controls	8	7	4	3
γ-rays	18 hours	6	6	2	4
	Controls	6	6	3	3

the γ-rays alone being operative. Under these circumstances, exposures lasting several hours were without any appreciable effect upon the subsequent proliferation of the tumours, and an exposure of eighteen hours was insufficient for a decided inhibitory action.

The experimental data in Table V. show that the β-rays are mainly responsible for the inhibitory effect upon the growth of the tumour when transplanted, an exposure to these rays lasting about one hour being sufficient to prevent any subsequent growth. For shorter periods the arrest of growth is not complete.

Although the experimental numbers are small, they show that irradiation for fifteen minutes is insufficient to prevent the growth of the tumour, but if extended to more than one hour there is complete arrest of growth. The results corresponding to thirty minutes' irradiation are of special interest, for although a certain percentage of the transplantations did

eventually develop into tumours, their growth was markedly delayed, as the following data contained in Table VI. show: —

TABLE VI.

Number of Mice.			Growth of Tumour.		Number of Days after Inoculation.
Inoculated.	Survived.		Positive.	Negative.	
6	5	Experimental	1	4	19
6	5	Control	3+2?	0	
6	5	Experimental	2	3	26
6	5	Control	5	0	
6	5	Experimental	4	1	34
6	5	Control	5	0	
6	4	Experimental	3	1	37
6	4	Control	4	0	

Thirty-four days subsequent to the inoculations, microscopical examination of one of the experimental growths left no doubt as to the presence of tumour cells.

The Mode of Action of the Radiations.

The experiments which have been described demonstrate that excised tumour tissue is affected by X-ray and radium radiations in such a way that its power of proliferation on subsequent inoculation is inhibited. This has only been thoroughly demonstrated in the case of one strain of tumour, but similar results have been obtained with a secondary sarcoma of the mouse, No. 100, A, Imperial Cancer Research, which has been exposed in an identical manner to soft X-rays for one hour, and β- and γ-rays for two and three-quarter hours.

The possibility that this phenomenon in the case of X-rays is due to heat can, with the precautions adopted, be disregarded. It has also been shown that the small amount of light given off by the bulb is not essential, interposing a black paper screen in no way altering the inhibitory action.

To assume that the tumour cells are actually killed by an adequate exposure to the radiations would provide a simple explanation of the results obtained, but it is difficult to obtain evidence that this is the case, and there is good reason to doubt the truth of the assumption.

It has been shown by one of us (Chambers and Russ, 1911) (4) that the phagocytic power of leucocytes is unaffected by considerably longer exposures than have been employed in these experiments to an equally intense source of β- and γ-rays, and a similar result has been obtained on exposing them to X-rays.

It has been frequently noticed that, macroscopically, the irradiated tumour has become more transparent than the control portion, but in sections which have been made and compared on several occasions no definite changes have been observed. The cell nuclei continue to stain well and karyokinetic figures are numerous, even in tissue which has been removed from the mouse and subjected to the action of β-rays from radium for several hours.

This has also been observed by Haaland (1910) (6), who exposed mouse tumour to radium (quantity not stated) for twenty-two hours in the ice-chest. He found that the immunising action of the tumour as well as its power of proliferation was abolished by such an exposure.

It was thought that a comparison of the behaviour of irradiated and control grafts at intervals after inoculation might assist in elucidating the mode of the inhibitory action. A number of mice were therefore inoculated with tumour tissue which had been exposed to β- and γ-rays for a time sufficient to ensure that no tumour would develop and simultaneously with control tumour. The mice were then killed at intervals, the grafts fixed, sections made and stained, and the appearances compared. No obvious increase in size was observed in the irradiated grafts. The appearances of the grafts may be seen in Plates I.–IV., in which are microphotographs of the irradiated and control grafts at various stages.

On the second day (Plate I., Figs. 1 and 2) the appearances are similar to those which have been described by several observers, and no essential differences can be made out. The greater part of the grafts appears necrotic, infiltrated with leucocytes, and there is a proliferation of fibrous tissue in the surrounding tissue. Both in the irradiated and control grafts some cells of the parenchyma, whose nuclei stain well, which appear to be alive though there is no evidence of active division, are seen at the periphery.

On the fourth day (Plate II., Figs. 3 and 4) there is still no striking difference between the two grafts. Karyokinetic figures are present in both.

On the sixth day (Plate III., Fig. 5) the control tumour is seen to be undergoing rapid proliferation, and the graft, though still small, has the appearance of the original tumour in miniature. The appearance of the irradiated graft (Plate III., Fig. 6) still resembles that of the control, but the cells of the parenchyma are more irregular in shape and there is more fibrous tissue present. Karyokinetic figures may be seen in both sections.

Eight days after inoculation (Plate IV., Fig. 7) the control tumour is the size of a hemp-seed, and karyokinetic figures are numerous. The irradiated graft (Plate IV., Fig. 8) consists almost entirely of fibrous tissue; a few cells of doubtful nature are seen, probably the last representatives of the parenchyma of the graft.

On the eleventh day the control tumour resembles that of the eighth. The irradiated tumour could not be identified with certainty; the sections obtained show only fibrous tissue.

It seems justifiable to conclude from these observations that the cells of the irradiated graft were not actually killed before inoculation, as they appeared to persist for several days, and even to undergo proliferation.

Some attempt has been made to demonstrate chemical changes in the irradiated tissue. In view of the statements that have been made about the action of radiations upon ferments, a comparative estimate of the amino-acids present in the irradiated and control tissue was made, both immediately after irradiation and after forty-eight hours' incubation, by Sörensen's method; but the results obtained have not demonstrated any certain differences between them, and this method appears unsuitable for the small amounts of material available.

Conclusions.

1. Freshly excised mouse tumours, when sufficiently irradiated by X-rays and subsequently inoculated into other mice, do not proliferate (p. 53).

2. The inhibitory effect is more marked when the irradiation is brought about by means of soft, i.e. easily absorbed, X-rays, than when a very penetrating type is used.

3. Excised tumours irradiated for about one hour by the β-rays from 5·6 mgrm. of radium bromide do not proliferate on transplantation. An exposure of eighteen hours to the γ-rays from the same quantity of radium is insufficient for an appreciable inhibitory action.

4. Histological examination of irradiated grafts at intervals after inoculation shows that the cells of the parenchyma of the tumour persist for several days, but are eventually replaced by fibrous tissue.

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DESCRIPTION OF PLATES.

FIG. 1.—Irradiated graft, second day, showing central necrosis, with persistent tumour cells at periphery.

FIG. 2.—Control graft, second day. Similar appearance to 1.

FIG. 3.—Irradiated graft, fourth day. Tumour cells undergoing proliferation.

FIG. 4.—Control graft, fourth day, resembles 3.

FIG. 5.—Irradiated graft, sixth day, cells irregular in shape, and more fibrous tissue present than in control.

FIG. 6.—Control graft, sixth day. Development advancing.

FIG. 7.—Irradiated graft, eighth day. Very few tumour cells remain, further increase of fibrous tissue.

FIG. 8.—Control graft, eighth day. Appearance now that of fully developed tumour.

Plate I.

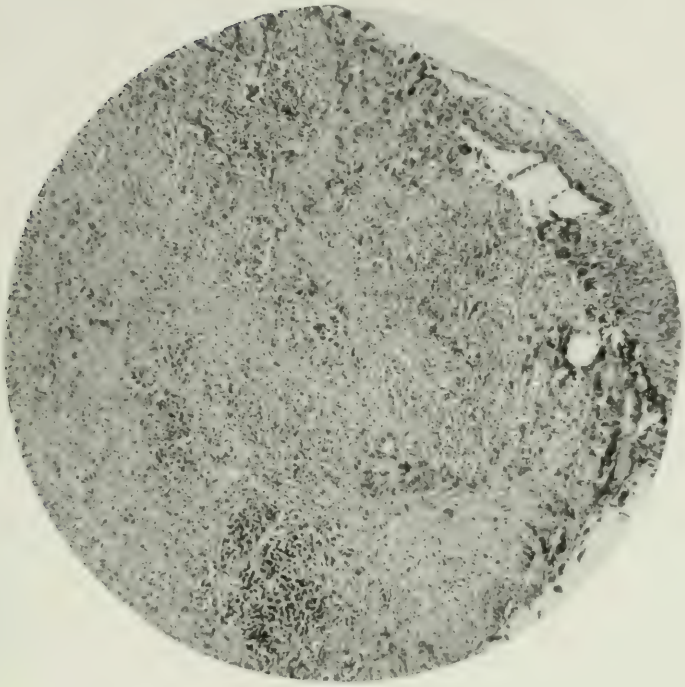


Fig. 1.

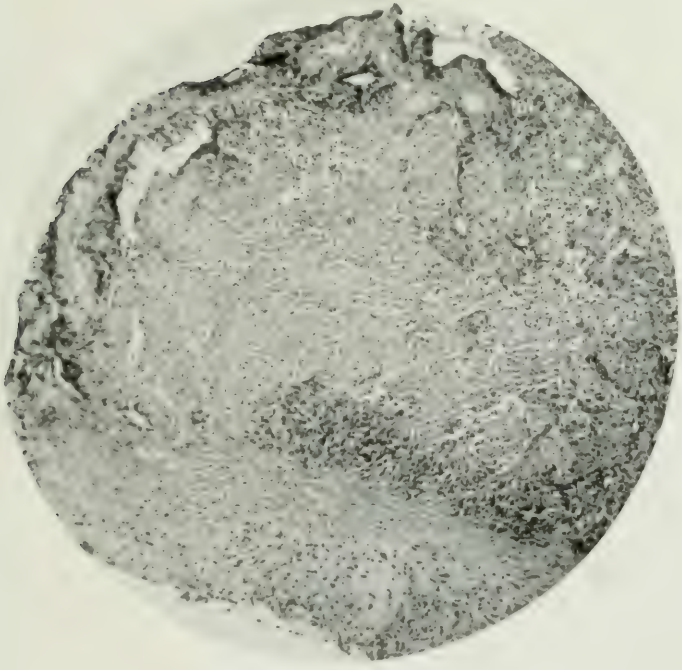


Fig. 2.

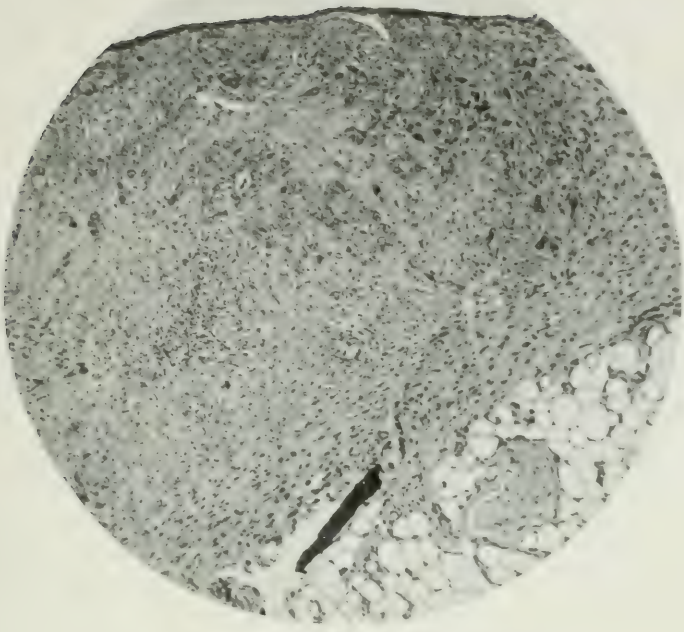


Fig. 3.

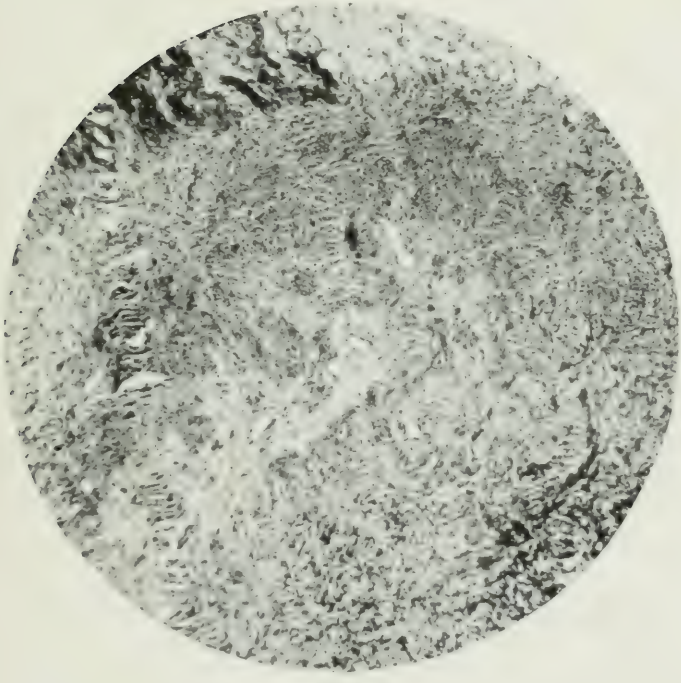


Fig. 4.

Plate III.

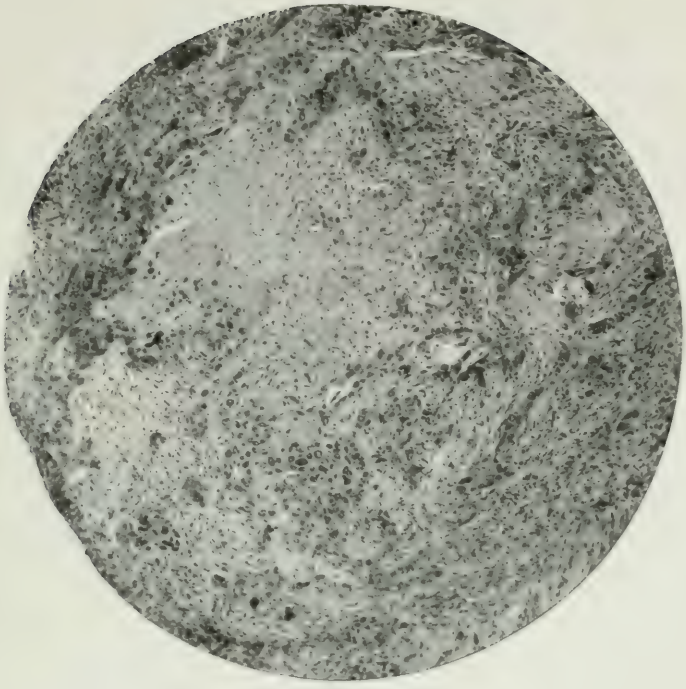


Fig. 5.

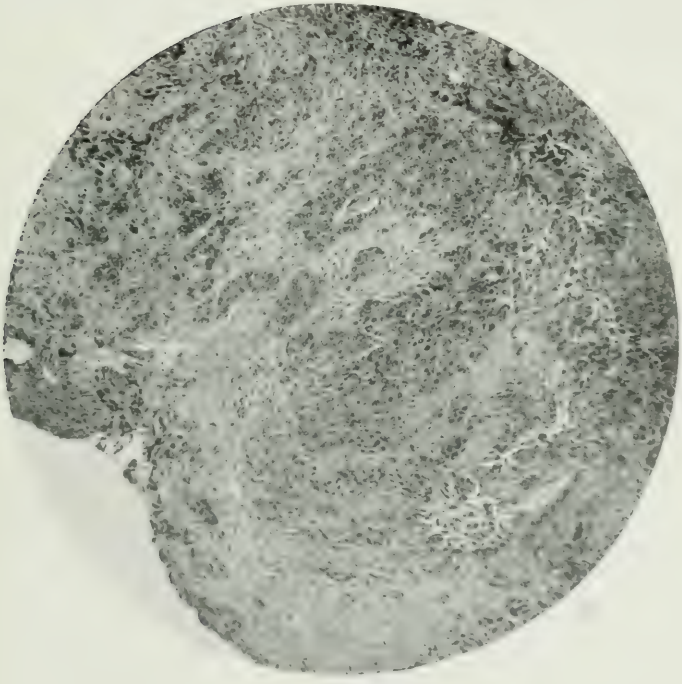


Fig. 6.

Plate IV.

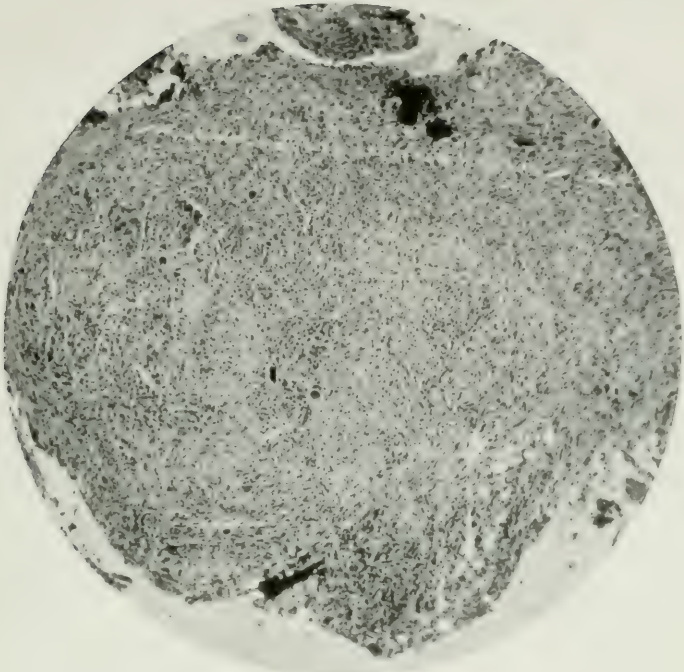


Fig. 7.

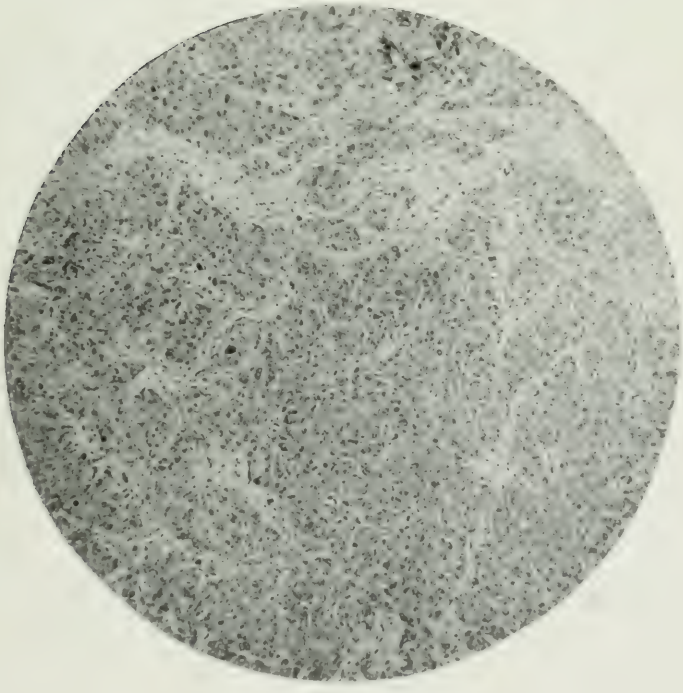


Fig. 8.

THE EFFECTS OF X-RAYS UPON VARIOUS ORGANIC SUBSTANCES.

By H. A. COLWELL and S. RUSS.

(*Communicated in part to the Physical Society of London, May 10th*, 1912.)

In view of the profound effects which are produced upon the animal body as a result of exposure to X-rays, it was decided to subject a series of organic substances to their action and then to determine what changes could be detected in the irradiated material. Both chemical and physical methods were employed in the investigation, and reference will be made to them when the individual substances are considered.

The substances hitherto examined are certain proteins and carbohydrates and the products of their hydrolysis. The protein series comprises blood serum, serum albumin, serum globulin, Witte peptone, alanin, and nucleo albumin; the carbohydrates were starch, glycogen, and cane sugar.

At the outset it may be stated that the two substances in which changes were detected as the result of irradiation were nucleo albumin and starch. The only change so far established in the nucleo albumin is a physical one; starch, however, shows chemical changes in addition.

Method of Experiment.

All of the substances, with the exception of blood serum, were examined in watery or saline solutions. Usually about 50 c.c. were placed in a Petri dish, sealed with a very thin sheet of mica to prevent evaporation and placed about 2 cm. below an X-ray bulb, worked under conditions giving a moderately soft type of radiation, the spark-gap being about 8 cm. The rise in temperature of the fluids, occasioned by the gradual warming of the bulb, never exceeded room temperature by more than a few degrees.

The fluids were irradiated for periods of from 2 to $8\frac{1}{2}$ hours and then examined.

Proteins.—*Serum, Serum Albumin, and Serum Globulin.* These were in all cases derived from fresh sheep's blood. To obtain the albumin and globulin, a sample of serum was treated with an equal volume of a saturated solution of ammonium sulphate, thus precipitating the globulin portion, the albumin remaining in solution. On the addition of water to the globulin precipitate it rapidly dissolved, giving a solution of serum globulin in dilute $(NH_4)_2SO_4$.

The albumin and globulin solutions were examined as soon as possible after irradiation, since protein solutions in the presence of $(NH_4)_2SO_4$ become alkaline owing to the liberation of free ammonia.

The periods of irradiation were as follows:—Serum, 2 hours; serum globulin and serum albumin, $1\frac{3}{4}$ hours. No difference was found in the irradiated and control samples either in appearance or viscosity; the method of determining the latter is detailed subsequently.

In the chemical examination of blood serum several samples of control and irradiated specimens were examined quantitatively by means of the biuret reaction. Although the method is not capable of such delicate application in the case of native proteins as in that of albumose or peptone, it affords a test of the breaking down of protein.

No change was detected in the irradiated serum.

In the case of serum albumin and globulin this procedure was not followed because the presence of $(NH_4)_2SO_4$, even in small quantities, causes an alteration in the tints produced by the addition of the alkali-copper mixture used in biuret estimations. In these cases, as well as in that of serum as a whole, the following procedure was adopted:—The control and irradiated fluids were dialysed for twenty-four hours, when the dialysates were examined for some of the more readily detected disintegration products of protein, namely, cystine, tyrosin, and tryptophane. None of these was, however, detected in the dialysates.

Samples of the irradiated serum were examined for a possible change in its refractive index, but none was observed.

It therefore seems justifiable to conclude that two hours'

irradiation by X-rays under the conditions specified has no appreciable disintegrating effect upon protein, at all events in the directions in which the investigation has been carried out.

Witte Peptone.—A 10 per cent. solution was irradiated for two hours. No change in viscosity was observed.

In the case of albumose or peptone, the biuret method has been shown by Vernon and others to be capable of very delicate application. Repeated estimations by this method failed to indicate any chemical change as a result of the irradiation. Examination of the dialysates in the manner described confirmed this finding.

Alanin.—Exposure of a 5 per cent. solution to X-rays for two hours produced no apparent alteration.

Portions of the irradiated and control fluids were titrated with standard NaOH, phenolphthalein being employed as indicator; no differences were detected. The titrations were repeated with identical results with samples to which neutral formalin had been added. This addition has the effect of converting the amino group NH_2 of the alanin into NCH_2, with the result that the carboxyl (COOH) group is able to exert its full acid effect.*

Nucleo Albumin.—To prepare this substance lamb's thymus was minced, ground up with its own volume of sodium chloride, and treated with excess of distilled water. Some of the material which floated to the surface was dissolved in a 5 per cent. solution of sodium carbonate and a sample of the very viscous fluid irradiated. After two hours' irradiation a marked diminution in the viscosity was observed. This, however, was the only alteration detected. Titration against standard acid, dialysis and examination of the dialysates, quantitative biuret measurements and refractive index determinations of the irradiated and control fluids, yielded no differences between them, although carried out on several occasions.

Carbohydrates.—*Cane Sugar.*—A solution was irradiated for two hours. No alteration in viscosity was observed, neither was any conversion into reducing sugar detected. Polarimeter estimations gave identical readings for the irradiated and control fluids.

* *Vide* these Archives, vol. xix., p. 56.

Glycogen.—A 4 per cent. solution was irradiated for eight and a half hours. Examination was made for the presence of reducing sugar and for that of bodies having the character of dextrins, but with negative results.

Starch.—Solutions were prepared in the usual manner and contained 4 grams of starch in 250 c.c. of distilled water.

Physical Changes.

After periods of irradiation ranging in various experiments from 2 to 8½ hours, two changes in the fluid were manifest, namely, a marked diminution in its viscosity and a decrease in its opacity when compared with a portion of the original solution.

The viscosities of the experimental and control solutions were compared by means of an apparatus consisting of a horizontal capillary tube sealed to a vertical glass cylinder. The time taken for the liquid to fall between two fixed marks on the cylinder was measured.

After prolonged irradiation it was found that the viscosity of the starch solution was so much diminished that the rate of flow through the system was more than doubled, as may be seen from the data in the Table, which represents a typical series of observations.

TABLE.

Comparison of Viscosities.

	Control. Time of flow.		Experimental. Time of flow.	
Temp. 18·3°C.	5′ 02″		2′ 08″	Temp. 18·8°C.
	5′ 11″		1′ 59″	
	5′ 11″		1′ 57″	
	5′ 14″		1′ 58″	

A calibration of the instrument with three liquids of known viscosities showed that the reduction in time (about one-half) observed for the irradiated starch corresponded to a rather larger diminution in the viscosity than this number indicates. The change in viscosity and opacity suggested the possible conversion of the starch into some of its cleavage products,

and accordingly experiments were undertaken to determine what chemical changes, if any, had occurred as a result of the irradiation.

Chemical Changes.

The Iodine Reaction.—The control and irradiated solutions both gave a deep-blue colour with iodine.

No reduction was obtained on boiling with Fehling's solution or with alkaline safranin in the case of either the control or experimental fluid, showing that no conversion into reducing sugar had taken place.

Precipitation by Electrolytes.—It has been shown by Young* that starch and some of its early cleavage products are precipitable by certain electrolytes, among which are sodium sulphate and ammonium sulphate.

1. When a saturated solution of sodium sulphate is added to a solution of starch and left standing for some hours it precipitates the ordinary starch, but not soluble starch or dextrin.

Accordingly samples of the control and irradiated solutions were saturated with sodium sulphate and allowed to stand for some hours. A marked difference was observed in the two cases.

The control showed a well-marked precipitate, while the irradiated solution gave only a small precipitate and remained turbid.

After centrifugalisation, the supernatant fluids were pipetted off. That from the control solution gave no coloration with iodine, the irradiated giving a deep-blue colour. This showed that a portion of the starch had been converted into soluble starch, which is not precipitated by sodium sulphate.

2. A saturated solution of ammonium sulphate, when added to an equal volume of starch solution (i.e., half-saturation) and left for a few days, precipitates all the starch and any soluble starch present, but has not this action on dextrin.

To see whether this substance had been formed in the

* Young, "Journal of Physiology," xxii., p. 401 (1898).

irradiated fluid, samples of the control and irradiated fluids were taken, and to each was added its own volume of a saturated solution of ammonium sulphate. After being allowed to stand for two days the filtrates were examined. In the control no coloration was obtained on the addition of iodine, while the irradiated portion gave a port-wine colour indicating the presence of erythrodextrins.

Quantitative Estimation of the Dextrin Formed.

The procedure just detailed for the detection of dextrin in the irradiated starch solution was carried a stage further in order to obtain some idea of the percentage change occurring in the starch.

After irradiation for eight and a half hours the dextrin formed in the solution was separated from the starch and the soluble starch in the manner indicated. The ammonium sulphate remaining in the filtrate was got rid of by dialysis. This was continued for several days until no precipitate was obtained with barium chloride, thus showing the complete absence of ammonium sulphate. The pure dextrin left was evaporated down and weighed. Starting with 15 c.c. of starch solution (4 grammes in 250 c.c. of distilled water), which contained 0·24 gramme of starch, the dextrin obtained after irradiation weighed 0·0108 gramme. Hence nearly 5 per cent. of the starch had been converted into dextrin. Owing to the intermediate formation of soluble starch, this indicates that a considerably larger percentage of the starch had been altered.

The Action of X-rays on Dextrin.

Having shown the conversion of starch into soluble starch and then to dextrin it was decided to see whether the sequence of changes was continued and could be detected at any further stage, possibly to the achroodextrins. A 5 per cent. solution of commercial dextrin was obtained and irradiated for eight and a half hours in the manner already described.

Subsequent to the irradiation no change in the appearance or in the viscosity of the fluid was observed.

Two Nessler glasses were taken, and in each were put 15 c.c. of water and 0·1 c.c. of iodine solution. To one was added 0·1 c.c. of the control and to the other 0·1 c.c. of the irradiated dextrin. No difference in tint was observed. On varying the quantities of iodine and dextrin the agreement of tint in the two cases was maintained.

Further, on saturating equal volumes of the control and irradiated dextrin with crystals of ammonium sulphate a small precipitate of erythrodextrin was obtained in each case, apparently equal in bulk on centrifugalisation.

The filtrates gave a red colour with iodine, of equal intensity when the comparison was made under similar conditions. No evidence was obtained therefore of any conversion of the erythrodextrins to the achroodextrins.

It is interesting in this connection to find that M. Massol* was able to convert starch into glucose by exposing it to ultra-violet light for long periods.

The Nature of the Action upon Starch.

It is well known that solutions of starch may gradually be converted into the erythrodextrins and achroodextrins by hydrolysis, and a simple explanation of the recorded results of irradiation might be put forward on these lines if hydrogen and hydroxyl ions were liberated from distilled water when irradiated by X-rays; but from the work of Kernbaum,† who exposed flasks of distilled water to the action of X-rays for 100 hours without any trace of gas being liberated, it seems clear that the part played by the water in the changes occurring in the starch solution must be a very small one and insufficient to account for them. The alternative is that the effect is due to a direct action upon the molecules of the starch caused by the X-rays or by the secondary rays (corpuscular or secondary X-rays) which may be produced by the primary X-rays in their passage through the solution.

* M. Massol, "Comptes Rendus," 152, p. 902.

† Kernbaum, "Comptes Rendus," Congrès International de Radiologie et d'Electricité, Tome I., p. 135.

The conversion of starch into dextrin is generally held to correspond to the change of a complex molecule into one of a more simple type (though still complex). Whether this actually corresponds to a more stable type, as the experiments upon dextrin seem to show, cannot be decided without more numerous agents being brought to bear upon the case and quantitative comparisons made of the effects observed. It is hoped that observations with different kinds of radiation may serve in this connection.

ON THE USE OF ELECTROSCOPES FOR THE MEASUREMENT OF RADIO-ACTIVITY.

By W. S. LAZARUS-BARLOW.

Since, in the two papers which follow, estimation of radium in animal tissues has been carried out, and since the emanation electroscope is necessary for the detection and estimation of the small quantities that may be present, it is perhaps advisable to describe the apparatus, to indicate the precautions that must be adopted in its use, and explain shortly how the results obtained by its means are to be interpreted. It is unnecessary to add that the present information is only laid before the non-physicist, and particularly before the investigator on biological subjects. For purposes of simplicity I shall assume a complete ignorance of the properties of radium and other radio-active substances in the reader.

On Radio-activity and Radiations Generally.—A substance such as mercury, iron, or gold has chemical properties, and the same is true of radium, thorium, uranium, actinium, ionium, &c.; but with these chemical properties, strictly so considered, we are not concerned. On the other hand, radium, thorium, uranium, actinium, and ionium possess certain definite electrical properties, owing to the fact that they are in a constant state of atomic disintegration, which do not exist or cannot be recognised in mercury, iron, or gold. It is in virtue of these peculiar electrical properties that radium, etc., are termed radio-active elements. The peculiar electrical property of these elements is a characteristic of the atom, so that all compounds and salts of radio-active elements are themselves radio-active in proportion to the amount of the radio-active base they contain.

The peculiar electrical property is manifested by the power of the substances in question to ionise a gas, that is, to split the molecules of that gas into their component ions. Under ordinary circumstances a gas (e.g. air) is electrically neutral because its electrically neutral constituent molecules

immensely predominate in numbers over the free (electrically charged) ions present. But in the presence of a radio-active substance with its disrupting power, a certain number of additional molecules of the gas are broken up and ions are set free, one-half of which carry a + electrical charge, and the other half a − electrical charge.

The degree to which this ionisation takes place in any particular gas is dependent upon several factors, the chief of which are the *kind* of the radio-active element present which induces the ionisation and the *amount* of the radio-active element present. For example, weight for weight radium induces enormously more ionisation than thorium, and 10 milligrams of radium produce ten times as much ionisation as one milligram. It follows that measurement of the ionisation indicates the amount of radio-active element present, provided that the nature of the radio-active element producing ionisation be known or can be determined, and provided that it can be made certain that the ionisation is actually dependent upon radio-activity and not upon some gross chemical or physical reaction such as evaporation of a fluid or combustion in a flame.

The actual factors upon which the possession of peculiar electrical properties by radio-active substances depends are (1) α-particles, which are shot off with an initial velocity of about 20,000 miles a second, which carry a positive electrical charge, and which after losing that charge have been proved to be helium atoms; (2) β-particles or negative electrons, far smaller than α particles, and moving with a velocity of about 200,000 miles a second; and (3) γ-rays, which travel at an even greater velocity and the nature of which is still uncertain. Not all these factors are possessed by each and every radio-active element; the Crookes' tube affords analogies with all the radiations from radio-active elements in some part or other of its system.

Now, in virtue of these α-particles, β-particles, and γ-rays more or less singly or in combination, according to the nature of their source, ionisation of a gas by means of a radio-active body or X-ray tube can be differentiated from ionisation due to gross chemical or physical changes such as those mentioned above. For α-particles, β-particles, and γ-rays, owing to their

velocity, can pass through a closed box and ionise the gas on the other side, whereas this property does not obtain in the other cases mentioned. Further, the α-particles, β-particles, and γ-rays can be differentiated from one another by the thickness of the screen they can traverse; γ-rays from radium can ionise a gas when separated from it by about 6 inches of lead, β-particles when separated by a millimetre or so of aluminium, but α-particles only when separated from the gas by an extremely thin sheet of mica or aluminium.

The degree of ionisation which can be effected by α-particles, β-particles, and γ-rays is not identical quite apart from the different thicknesses of the screens they can traverse. Thus each α-particle given off by a sample of radium ionises at least a thousand times as many molecules of air per unit time as each γ-ray given off by the same sample. Hence it follows that by utilising the ionisation occasioned by α-particles, one can detect and measure quantities of radium far less than one could if one relied upon using the γ-rays alone. It is this fact which explains the employment of the emanation electroscope, for by its means the ionisation caused by α-particles is measured.

The Principle on which an Electroscope is used.—Whatever variety of electroscope be used, the principle of its employment is as follows:—If an electrical charge be given to an insulated gold leaf, for example that in an ordinary gold-leaf electroscope of the student, that charge is gradually neutralised by the ions in the air of opposite charge which are always present, and are probably dependent for the most part upon ionisation caused by the radium distributed in the earth's crust. The rate at which this neutralisation of charge takes place under normal circumstances is known as the "natural leak," and it can be measured by observing the rate at which the charged gold leaf falls a certain distance. When the natural leak has been determined, if a specifically ionising agent, e.g. radium, be brought into the neighbourhood of the charged gold leaf, the number of ions carrying an opposite charge that are attracted to the gold-leaf system in unit time is increased, neutralisation of the charge on the gold leaf is more rapid, and the rate of leak is increased. On removal of the ionising agent (radium), re-combination of the

free ions in the gas takes place forthwith, and the rate of leak again reverts to the "natural leak." It follows that accurate determination of the natural leak is a matter of the greatest importance, and the importance of accuracy increases as the amount of radium or other radio-active element with which one is dealing diminishes. Indeed, it may be said that estimation of minute quantities of radium essentially consists in accurate determination of natural leak. The electroscope is therefore used simply as a delicate indicator of ionisation much in the same way as litmus is used as an indicator for acidity or alkalinity. Provided the natural leak be known, it can be determined with considerable accuracy whether a substance does or does not increase the rate of leak, i.e. ionise the air within the electroscope, by how much it increases the rate of leak, and therefore, provided the other precautions are taken, whether the substance is radio-active and to what degree.

The emanation electroscope as indicator of the *nature* of the radio-active element.—So far it has been shown that the electroscope can be used as an indicator of radio-activity and a measure of radio-activity; but it can in addition be used to indicate the nature of the radio-active element, and by comparison with standards to indicate the quantity of the radio-active element present. This depends upon the fact that the products of disintegration of radium, for example, are different from those of thorium, thorium from actinium, and so forth. And the differences that are made use of for the purpose under consideration are those which concern the "life" and "decay" of the various disintegration products. Thus, it is known that radium forms as its first disintegration product a chemically inert gas (probably belonging to the argon series) known as radium emanation, and that thorium forms similarly a thorium emanation. In each case the emanation is capable of ionising air, and therefore accelerating the electroscopic leak, owing the fact that it is itself in process of disintegration. But whereas half of the total disintegration change obtaining in the case of radium emanation takes 3·86 days to be brought about, half of the total disintegration change obtaining in the case of thorium emanation is accomplished in fifty seconds. And since this decay goes on in geometrical

progression the rate of decay is an important indication (often the only one) of the identity of the radio-active substance under investigation. The following is an example. Suppose that the natural leak of the electroscope be ·2 of a division per minute, and suppose that we have in a flask a radio-active emanation capable of causing a leak of 10 divisions per minute. Say that it will take five minutes to transfer the emanation to the electroscope. Then if the emanation be that given off by radium with its half-decay period of 3·86 days the five minutes will be negligible; on introduction of the emanation within the electroscope we shall obtain a leak of 10 divisions per minute. But if the emanation be that given off by thorium with its half-period of fifty seconds, the five minutes is not negligible. For whereas by hypothesis the thorium emanation gives a leak of 10 divisions per minute in the *flask*, it will have been reduced by one-half six times in geometrical progression during the five minutes' delay necessary to transfer it from flask to electroscope.

At 0 min.	0 sec.	it gives a leak of	10	divisions	per minute.
,, 0 min.	50 sec.	,,	5	,,	,,
,, 1 min.	40 sec.	,,	2·5	,,	,,
,, 2 min.	30 sec.	,,	1·25	,,	,,
,, 3 min.	20 sec.	,,	·625	,,	,,
,, 4 min.	10 sec.	,,	·3125	,,	,,
,, 5 min.	0 sec.	,,	·15625	,,	,,

Hence by the time the thorium emanation has been transferred from flask to electroscope its influence on electroscopic leak has practically disappeared.

But the emanation electroscope affords more than presumptive evidence as to the presence of radium, and again this depends upon the life and decay of the products of disintegration of the radium emanation itself.

Directly radium emanation is introduced within the electroscope it begins to form Radium A; Radium A begins to form Radium B; Radium B begins to form Radium C.* Now, the four substances Emanation, Radium A, Radium B,

* RaA + RaB + RaC together are termed "the active deposit of quick change," or shortly, "active deposit."

and Radium C, though all of them radio-active and therefore capable of ionising the air within the electroscope, are so in different degrees, and have different half-periods of decay. At starting there is only radium emanation in the electroscope, which gives off a certain number of α-particles and occasions a certain rate of leak. But the RaA which the RaEm forms also gives off α-particles, and the sum of the effect of each of these is shown by an increasing acceleration of the electroscopic leak until all the RaA has been formed (about 15 minutes after introduction of the gas into the electroscope). The period between the 15th and 30th minutes after introduction of the gas into the electroscope corresponds to the formation of RaB from RaA, and since RaB for our purposes exerts little or no ionising power, the rate of leak remains at a constant level. At the end of the first half-hour after introducing the gas into the electroscope the formation of RaC (with its α, β, and γ rays) from RaB begins to make itself felt on the rate of leak by a further increase of the already existing acceleration, and this further increase continues to quicken the rate of leak until the end of the third hour after introduction of the gas into the electroscope. From that time the leak gradually becomes slower until at the end of 3·86 days it is one-half that which obtained at the end of three hours.

The behaviour of radium emanation as described in the above paragraph is absolutely characteristic, but yet an additional proof that one is dealing with radium is obtained by observing the behaviour of the electroscopic leak after removal of the emanation. Since the emanation is a gas, it can be removed by repeated exhaustion of the reservoir of the electroscope. But the active deposit which the emanation has formed is a solid, and is deposited on the interior of the electroscope reservoir, and continues to give off its α, β, and γ rays in the formation of Radium D, which does not concern us owing to its relative inactivity. This active deposit cannot be removed by exhausting the electroscope, but must be allowed to decay naturally. And again the curve which is formed after exhaustion of the electroscope is characteristic. For the leak after exhausting the electroscope of the emanation it has contained is markedly less rapid than when

emanation was present, but is nevertheless more rapid than the natural leak. Successive estimations show a slower and slower leak, until by the end of three or four hours after exhausting the electroscope the active deposit has decayed

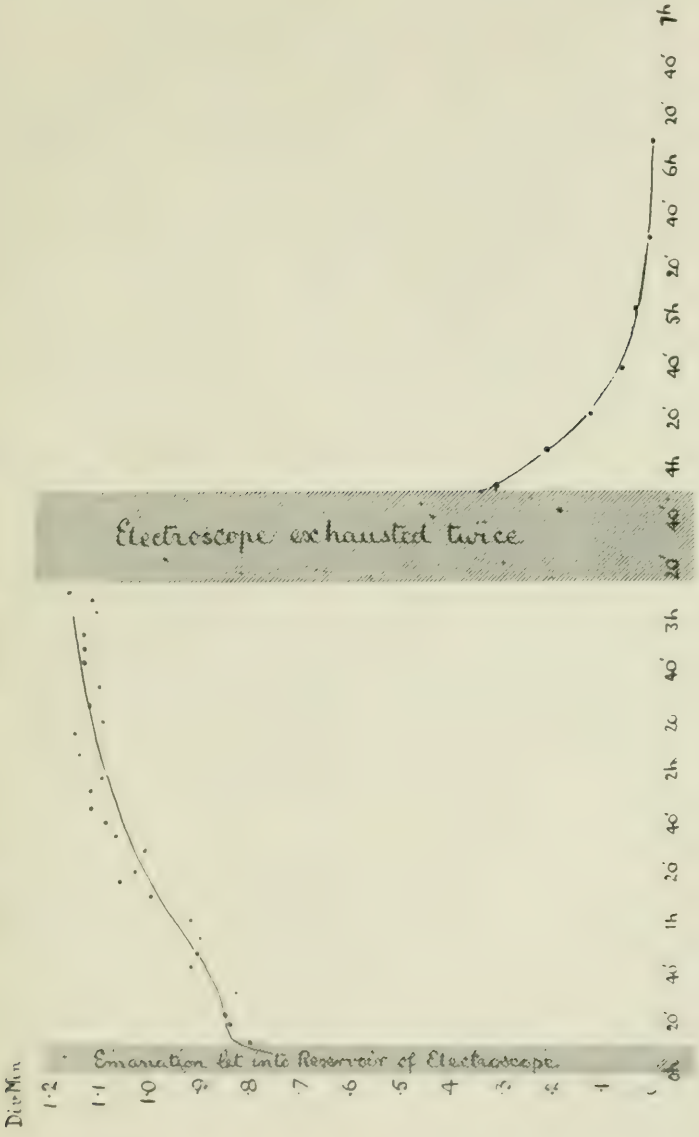


FIG. 1.

Leak given by Substance 300 compared with typical Curve of Radium Emanation.

into RaD to such an extent that its presence is no longer recognisable, i.e. the natural leak is again reached.

It must not be supposed that in actual practice the results indicated in the last paragraph but one obtain with the mathematical precision that has been set down, at all events in the hands of one who is not a skilled physicist. This is shown by the values actually obtained in the experiment represented by Fig. 1, which range on either side of the typical curve. Moreover, when the amounts of radium present are about the lower limit of recognition, i.e. when the experimental leak does not differ very greatly from the natural leak, experimental error becomes greater, the flattening of the curve corresponding to the formation of RaB disappears, and one is dependent upon 1° the immediate occurrence of a more rapid leak on introduction of the gas into the electroscope, 2° the gradual increase in rate of leak to a maximum at the end of the third hour, 3° a more rapid leak than the natural leak, but a less rapid leak than with the emanation within the electroscope which obtains after exhausting the electroscope, and 4° a slowing of the leak in successive observations, until by about three hours after exhausting the electroscope the initial natural leak is again reached. This succession of events is absolutely characteristic of radium emanation, and by a comparison of the maximum leak (less the natural leak) with the maximum leak observed in experimenting with a known quantity of pure radium (less the natural leak during that experiment), the amount of radium which produced the emanation in the first instance can be calculated. These points are shown by the curve obtained from an actual experiment (Fig. 1).

It is clear that the experiment as described essentially consists in the estimation of the amount of radium emanation present in a substance ; and though it would be certain, if it were found, that radium *emanation* was present in that substance, it would not follow that *radium itself* was present. For the emanation being a gas and radium being widely distributed, the substance might contain occluded gas though destitute of the radium which produced it. Thus pure distilled water contains no radium, but if it be kept in a room

in which there is radium emanation a certain proportion of the radium emanation in the air of that room will become dissolved in the distilled water and would evidence its presence if the water were examined by means of the emanation electroscope by the series of changes in leak that has been described.

It is possible to eliminate this source of error owing to two facts. First, the solubility of radium emanation is dependent upon temperature, and by boiling a fluid all dissolved emanation is expelled. Hence, though the distilled water referred to in the last paragraph would show the presence of radium emanation if it were examined without boiling, it would show the complete absence of radium emanation if it were thoroughly boiled before electroscopic examination. Second, the amount of radium emanation formed at any time is a constant function of the amount of radium present. In other words, radium "grows" radium emanation in a specific manner. This rate of growth is shown in the accompanying curve (Fig. 2), where it is seen

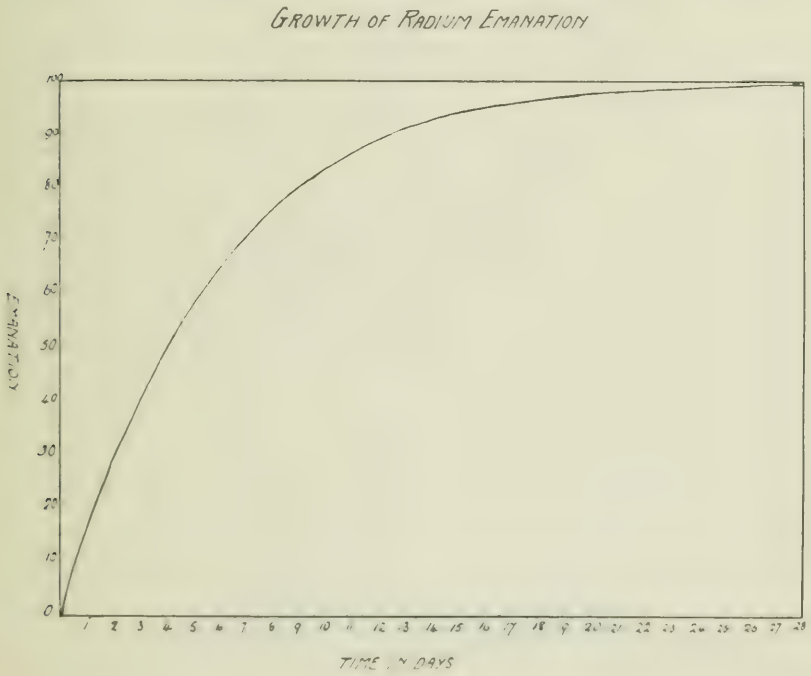


FIG. 2.

that by the end of four weeks almost 100 per cent. of the maximum amount of emanation associated with any given quantity of radium has been produced. Hence, if a solution under investigation be thoroughly boiled to expel any dissolved emanation, be then securely sealed and set aside for four weeks, any emanation that may be found in the gases expelled by boiling the solution at the end of four weeks must have been formed by radium itself within the solution during the period of four weeks. I assume here that the radium is not in the glass of the flask or other part of the apparatus, sources of error which when dealing with minute traces of radium need to be directly eliminated.

Description and Comparison of Electroscopes used.—In Figs. 3 and 4 the apparatus used in the estimation of radio-

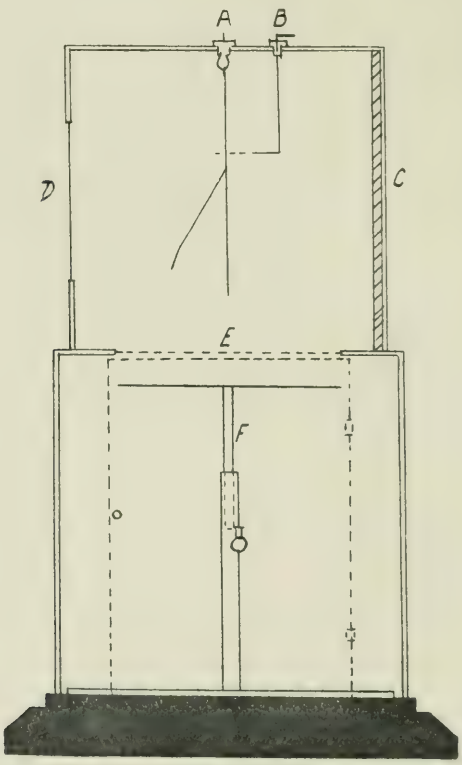


FIG. 3.

activity are diagrammatically represented. In Fig. 3 it is seen that by placing the substance on the platform (F) in the lower half of the electroscope the presence of α, β, and γ rays can be detected *; by placing the substance outside the electroscope but on the aluminium side (D) the β and γ rays can be detected, and by placing it outside the electroscope but on the lead and brass side (C) the γ-rays can be detected alone. A is the sulphur insulation and B the charging rod. So far as measurement of α-radiation from a solid substance is concerned, this electroscope is limited by the fact that the only α-particles that can be measured are those on its very surface: α-particles formed by the substance in its deeper layers cannot penetrate the screen afforded by the superincumbent layers. This is true to some extent even if the substance be a fluid. Nevertheless, a rough idea can be obtained of the α-particle value of any substance, and thus an idea can be formed of the amount of substance it is advisable to put up for estimation by means of α-particles with the emanation electroscope. Speaking broadly, if the rate of leak per minute be doubled owing to the presence of the substance on the platform of this electroscope, 3 gm. of the substance will give a satisfactory experiment when examined by means of the emanation electroscope. When the rate of leak is not appreciably altered, it is desirable to put up for emanation as much of the substance as can be obtained; in one instance recorded in the protocols 520 gm. were used.

The emanation apparatus (Fig. 4) essentially consists of two parts: 1° that part designed for boiling off and drying the gas; 2° the electroscope proper. These can be shut off from one another by taps, but it is possible to exhaust the whole or a part of the apparatus by means of the water exhaust pump.

The emanation electroscope itself consists of a closed metal chamber to contain the gas under investigation (DD) into which projects the lower three-quarters of an insulated metal rod. The upper quarter of this metal rod stands outside the gas chamber, and carries a strip of gold leaf (G). Since the

* The metallic grating which divides the electroscope into two approximately equal portions is to ensure that the electrical capacity of the gold-leaf system shall be constant.

rod is insulated an electrical charge can be given to it and to the gold leaf, causing the latter to stand out at an angle. The leaf is protected from air currents and temperature changes by being enclosed in a metal box having double walls with cotton wool between and mica-shielded windows for observation of the movements of the gold leaf. The leaf is observed through a telemicroscope magnifying about 10 diameters and carrying a scale with divisions in the eye-piece.

If the gas within the chamber (*DD*) contain a substance which is emitting α-particles these ionise the air in the vessel, and a portion of the charge on the charged metal rod and gold-leaf system gradually becomes neutralised. As neutralisation proceeds, the gold leaf more and more tends to assume the vertical position demanded by gravity, and the rate at which it does this is indicated by the rate at which the image of the gold leaf passes over a fixed portion of the scale in the telemicroscope.

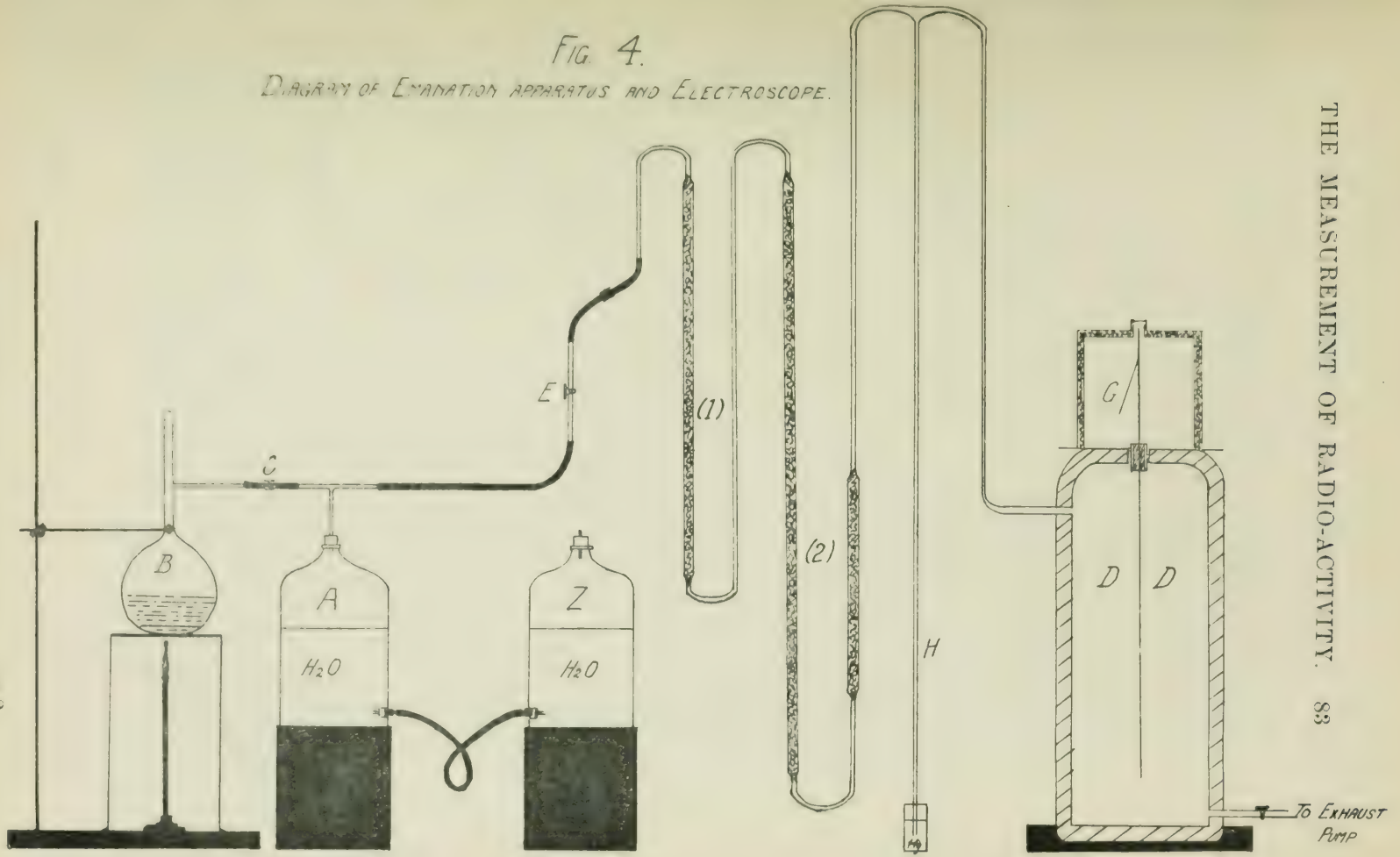
An experiment by means of the emanation electroscope consists of the following steps.

Having got the substance to be examined in solution, it is placed in a distilling flask (*B*), well boiled, and, after cooling, the neck and side-piece of the flask are sealed in the flame. The flask is then set aside for four weeks for any contained radium to grow its emanation. At the end of that time the actual determination by means of the emanation electroscope is carried out as follows :—

After determining the rate of natural leak with all taps closed and the whole apparatus at atmospheric pressure, the air in the glass gas reservoir (*A*) is expelled two or three times to ensure its freedom from emanation at the commencement of the experiment, the flask (*B*) is connected with the apparatus by means of the rubber attachment (*C*); the sealed end of the side-piece of the flask is broken off within the rubber tubing, and the contents of the flask are gradually raised to boiling point. During this time the screw-clamp on *C* is of course open. At the same time the reservoir of the electroscope (*D*) is being exhausted or partially exhausted (as shown by the Hg manometer *H*), the tap *E* being closed. After the flask has been vigorously in boiling for five minutes, the

FIG. 4.

DIAGRAM OF EMANATION APPARATUS AND ELECTROSCOPE.



screw-clamp C is closed while ebullition is going on. The tap E is now cautiously opened, and the rise of the fluid in the gas reservoir (A) indicates that the gas is being transferred over the $CaCl_2^1$ drying tubes (1 and 2) to the reservoir of the electroscope (D). When the fluid has risen to the neck of A the tap E is closed, the time is taken, the rubber tubing is disconnected from the side-piece of the flask, and the screw-clamp C is opened. Air passes into the gas reservoir till the fluids in A and Z are again at the same level, when the screw-clamp is closed, the tap E is cautiously opened, and again the gas reservoir is emptied into the reservoir of the electroscope. This is done a third time, and finally atmospheric pressure is established within the reservoir of the electroscope by allowing the air to pass in a gentle stream through the rubber connection. All taps are now closed, the time is again taken, the electroscope is charged to the initial point, and estimation of the experimental leak is begun. Repeated observations of leak are made over the same range of the scale for a period of three hours. The reservoir of the electroscope is then exhausted as fully as possible and refilled with dry air twice (or oftener if the amount of emanation has been great), and the leak repeatedly determined, until the initial natural leak has been reached. In the case of an experiment which goes well and indicates 1×10^{-7} mgr. Ra the whole procedure will have taken about eight hours.

The object of taking the time on completion of the first transference of gas from gas reservoir to reservoir of electroscope is in case the experimental leak is very rapid. If that be so it will be possible to estimate the amount of radium from the first level period of the emanation curve (second 15 minutes after introduction of the gas into the electroscope), whereby much time is saved.

From a consideration of the preceding paragraphs, it will be seen that the ordinary electroscope and the emanation electroscope are applicable in different directions, and afford different types of information. The ordinary electroscope informs us whether radio-activity is present or not in any substance in large quantity, and by a special arrangement (Fig. 3) it is possible to determine whether a radio-active substance gives

off α, β, or γ rays, or what combination of them. It does not, however, tell us the identity of the substance, though if, for example, a minute trace of a substance gives evidence of possession of a considerable amount of γ-rays, we shall probably be right in concluding that that substance contains radium, and not one of the other radio-active elements. And further, it is always possible, when a substance is directly introduced within the electroscope and is not separated from the gold leaf by a screen impermeable to gases, that any ionisation as evidenced by increased rate of leak may be due to gross chemical or physical, as distinguished from radio-active, changes. This objection hardly arises when testing the effect of β or γ rays; and, finally, an amount of a radio-active substance which might be readily determinable if examined in the emanation electroscope and by means of its α-particles, would be quite undetectable by the ordinary (or γ-ray) electroscope.

On the other hand, though the emanation electroscope will detect far smaller quantities of a radio-active substance, and by the shape of curve afforded by successive observations of the rate of leak may safely be used to determine the nature of the radio-active substance, it is only applicable in the case of those radio-active substances which form an emanation or gas as a part of their disintegration and, in medical research at all events, will probably find its greatest or only use in detection of and estimation of radium.

Range of applicability of the Ordinary and the Emanation Electroscope in Radium Estimations.

Since the essential factor upon which attention is focused in determining the presence or absence of radio-activity is whether the charged gold leaf loses its electricity (i.e., falls to the vertical uncharged position) more rapidly than normal or not, the first essential is to obtain the range of error of observations of normal leak. Reference to this point is made later (p. 88), but it may be said generally that the experimental error in determining leak cannot be brought lower than about 10 per cent., even with very great care, by the

medical researcher at all events, and with present apparatus. Hence, if a number of observations be made in the case of a leak which is actually one division on the scale in 10 minutes, values will be obtained varying between one division in 9 minutes and one division in 11 minutes, though the greater number will be round about one division in 10 minutes. Consequently absolute reliance can only be placed on experimental observations differing from the mean by more than 10 per cent. That is to say, if the mean natural leak of the ordinary electroscope be one division in 10 minutes, and the mean experimental leak give one division in 9 minutes, a radio-active substance is probably present; if the experimental leak give repeatedly one division in 8 or fewer minutes, a radio-active substance is (subject to considerations of general accuracy) present with certainty.

Now, in the case of the ordinary electroscope and testing by means of γ-rays this lower limit corresponds to about ·001 milligram of radium; amounts less than this cannot be measured with accuracy, though suspicion may be raised as to the presence of radium. In the case of the emanation electroscope, because we are utilising α-rays with their far higher ionising power as the indicator, the lower limit is greatly reduced. A 10 per cent. increase in rate of leak in this instance is detectable in the presence of the emanation produced at the end of four weeks by so small an amount as ·000000001 mgr. radium (1×10^{-9} mgr.). As will be seen by reference to the protocols at the ends of the two succeeding papers, the leak has been determined over a distance of five divisions on the scale. If the natural leak be such that it takes 50 minutes for the leaf to travel these five divisions, and the experimental leak such that it takes 44 minutes to travel over the same distance after recharging the electroscope to the initial point, then (*cæteris paribus*) it is certain that this increased rapidity is beyond the range of experimental error, and by calculation corresponds to about ·000000001 mgr. (1×10^{-9} mgr.) radium.

The upper limit set to use of the emanation electroscope is that due to an extremely rapid rate of leak. Where the rate is 100 divisions in 4 seconds, as in some of the experiments I have given elsewhere (p. 126), accuracy is quite

impossible; and though one can diminish the rate of leak by working at a diminished air-pressure, as will be indicated later (p. 89), a rate corresponding to 20 divisions in 12 seconds is, in practice, the most rapid that can be fairly relied upon. Working at atmospheric pressure, such a rate of leak corresponds to about ·00001 mgr. (1×10^{-5}) Ra. The optimum range for the emanation electroscope is 1×10^{-6} to 1×10^{-8} mgr. Ra. When, from a preliminary examination, it it is probable that the substance under investigation contains an amount of radium of that order or less, the emanation electroscope may be used with advantage—indeed, is a necessity; but when it is probable that a greater amount of radium is present, either the γ-ray electroscope should be used or the amount of substance set up for emanation in a flask for four weeks should be correspondingly diminished. To ensure accuracy both methods should be adopted.

Practical points in the use of Electroscopes.—It may be well to append some of the more important practical points in the use of electroscopes which I have learned by experience during the past six years, for the benefit of the medical investigator who wishes to work with them.

1. *Insulation.*—For the ordinary electroscope a sulphur bead insulation has been used. From time to time it needs careful scraping with a clean knife, and under all conditions the greatest care should be taken not to touch it with fingers. For the emanation electroscope sulphur is not so satisfactory, since the HCl gas introduced into the chamber after a time leads to the formation of a conducting black film on the sulphur (probably a sulphide of iron or copper according to the nature of the walls of the reservoir of the electroscope), and the insulation breaks down. Moreover, it is more difficult to obtain an air-tight joint; and this is important, since the reservoir is frequently exhausted to a high degree. For this electroscope, a glass tube passing through a vulcanite plug and separated therefrom by sealing wax, and carrying the metal rod embedded in sealing wax, has been found to work admirably for nearly a year. The vulcanite plug screws into the metal gas reservoir of the electroscope, and by means of a thick rubber washer a good air-tight joint is produced.

2. *Charging and re-charging the electroscope.*—When the electroscope is charged after a period of rest (say, the first thing in the morning) the leak that obtains is to a certain extent into the air, but far more into the insulation. Until the insulation has reached a "saturation" point for the particular range of the scale (i.e., voltage) at which one is about to work, observations on natural leak are fallacious. Thus the first determination of natural leak might give ·5 division on the scale per minute, the next determination ·4, then ·3, ·2, ·15 division per minute in an apparatus for which the natural leak is known to be very closely ·1 division a minute. Three hours may easily be occupied under these conditions in determining the true natural leak for the experiment about to be performed. To overcome this difficulty, repeated charging and discharging in the presence of radium has been resorted to, but the most satisfactory method has undoubtedly been to *never allow the electroscope to become discharged*, and to keep the charge as close to the range at which one will be working as possible. A little experience will tell one, if the working range be, say, 70–65 on the scale, how far the leaf must be charged at night or on Saturday to have leaked not lower than to about 55 on the scale by the following, or the Monday, morning. In this way much better results can be obtained, but even this method is not fully satisfactory. An electroscope which has been charged all night and has fallen to 55 will not give the same leak for the distance 70–65 as one which has only fallen to 90, on specifically charging to make the experiment. Owing to the difference in potential of the insulation and the gold leaf, either part of the charge on the insulation will pass to the gold leaf, giving an abnormally long natural leak, or part of the charge on the gold leaf will pass to the insulation, giving an abnormally short natural leak. Taking the same observation distance—70–65th division—this distance might easily be travelled over by the gold leaf in 35 minutes if the leaf stood at 55 before recharging to make the observation, or 50 minutes if the gold leaf stood at 65 before recharging, or 75 minutes if the gold leaf stood at 90 before recharging. Hence the first observation of natural leak, even though the electroscope have been charged all night, must not be taken unless a second coincides with it

closely. So great is this difficulty, that it is desirable to keep the leaf constantly charged to a definite potential, except during actual experiment, by means of storage cells.

Owing to the diminished ionisation that takes place at diminished pressure (there being less air to ionise), it is easy in the case of the emanation electroscope to keep the leaf charged for two days by exhausting the reservoir as far as possible. In the case of the ordinary electroscope this can only be done by charging the leaf till it stands at a right angle with the support, and then only if the insulation be so good that the leak is about ·1 division a minute.

The pressure at which observations are made.—It is naturally easier to carry out observations at atmospheric pressure, if only for the fact that the natural leak is more rapid the more air there is to ionise, and therefore a closer approximation to accuracy can be made. But since the amount of ionisation (*ceteris paribus*) depends upon the amount of air in the electroscope one can often obtain readings within the accurate range by working at a low pressure, whereas they would be outside accurate range if taken at atmospheric pressure. Thus a rate of leak of 20 divisions in 3 seconds at 760 mm. Hg pressure—which is outside the accurate range—would be $\frac{760}{190}\times 3=12$ sec.—within the range of accuracy—if the pressure within the reservoir of the electroscope were 190 mm. Hg, or $\frac{760}{95}\times 3=24$ sec. if the pressure within the electroscope were 95 mm. Hg. It follows that when a solution is suspected to contain a relatively large amount of emanation the electroscope should be exhausted as completely as possible, and the experimental readings taken at as low a pressure as possible. Correction can easily be made at the end of the experiment. It is mainly on this account that it is necessary for the whole apparatus to be thoroughly air-tight, so that the pressure shall remain steady throughout the experiment.

Clearing the apparatus of traces of emanation.—It is obvious that if emanation be present in the glass gas reservoir before boiling the flask a substance actually devoid of radium may appear to have grown emanation. This

experimental error is particularly likely to occur after a substance containing much radium has been examined, and for one of two reasons. Either a certain quantity of the emanation has remained dissolved in the water of the reservoir, or, in boiling, traces of the radium-containing solution in the flask have been carried over. If the water in the reservoir be boiled immediately before the experiment this difficulty can be overcome, but there is less risk of accident, and the result is as satisfactory if the air in the reservoir—which is ultimately to be passed into the reservoir of the electroscope—be replaced two or three times with fresh air immediately before the experiment by raising and lowering the level of the water.

NOTE.—Electroscopes of either kind described above may be obtained from C. W. Cook, University Works, Bridge Street, Manchester; a useful form of charger for the gold leaf (designed by Mr. C. E. S. Phillips) is made by A. C. Cossor, Farringdon Road, London.

ON THE PRESENCE OF RADIUM IN SOME CARCINOMATOUS TUMOURS AND OTHER TISSUES.

By W. S. LAZARUS-BARLOW.

(*First Communication.*)

(A portion of the present research was communicated to the Royal Society on March 14th, 1912.)

In the Fifteenth Volume of these Archives (Eighth Cancer Report, 1909, p. 126 and foll.) I brought forward evidence that acceleration of electroscopic leak may occur when carcinoma tissue, after extraction with acetone or with ether and subsequently with water, is introduced within an electroscope. The results have been criticised in respect of the smallness of the differences observed and of the possible alteration in capacity of the electroscope occasioned by introducing the various substances into it.

To meet the last objection an electroscope of small constant capacity has been designed (Fig. 3, p. 80). It consists of a brass box (100 mm. × 100 mm. × 100 mm.), the bottom of which is composed of wire gauze of narrow mesh. This box is securely seated on another brass box without top and provided with a well-fitting door to facilitate the introduction of various substances into the electroscope. The substances are placed on a metal platform which can be adjusted at various heights. Replacement of one side of the upper box by thin aluminium permits the measurement of β-radiations to be carried out, while reinforcement of the brass on the opposite side with 3 mm. of lead allows γ-rays to be measured.

Using this instrument 42 samples of various non-malignant and malignant tissues, after extraction and in a dry state, were examined as to their influence on leak. The substances were as follows: Three specimens of normal liver, two of normal lung, two of primary sarcoma, eight of secondary carcinoma, and twenty-seven of primary carcinoma. All the specimens were from the human subject, and the diagnosis was made microscopically.

The natural leak was determined with a disc of white cardboard on the metal platform, and the substances were

placed on a similar cardboard disc. The distance of the platform from the wire gauze was kept constant throughout the entire series of observations. The value given for each substance is the mean of 10 observations as compared with the mean of 11 alternating observations of the natural leak. The results were as follows :—

	Ref. No.	Substance.	Leak of Substance (Natural Leak=1)
Non-malignant	378 ...	Liver, normal human	1·07
	386 ...	,, ,, ,, ...	1·01
	*437 ...	,, ,, ,, ...	·94
	119 ...	Lung ,, ,, ...	1·02
	125 ...	,, ,, ,, ...	1·04
	39 ...	Primary sarcoma of adrenal	1·06
	87 ...	,, ,, femur	·96
	433 ...	Lymphatic glands secondary to carcinoma of rectum	1·14
	148 ...	,, ,, ,, ,, breast	·97
	792 ...	,, ,, ,, ,, ,,	1·09
	*440 ...	,, ,, ,, ,, ,,	1·29
	434 ...	Hepatic metastasis ,, ,, rectum	1·14
	576 ...	,, ,, ,, ,, ,,	·94
	338 ...	,, ,, ,, ,, breast	1·06
	790 ...	,, ,, ,, ,, ,,	1·02
	407 ...	Primary carcinoma of bladder	1·04
	94 ...	,, ,, cervix	1·07
	548 ...	,, ,, ,, ...	·85
	697 Sample A	,, ,, ,, ...	1·01
	,, B	,, ,, ,, ...	1·02
*	,, C	,, ,, ,, ...	21·28
	,, D	,, ,, ,, ...	1·71
	,, E	,, ,, ,, ...	1·14
	,, F	,, ,, ,, ...	1·10
*	,, G	,, ,, ,, ...	1·06
	71 ...	,, ,, ovary	1·02
	794 ...	,, ,, ,, ...	1·06
	117 ...	,, ,, tongue	1·03
	*793 ...	,, ,, ,, ...	2·24
	575 ...	,, ,, rectum	1·11
	768 ...	,, ,, ,, ...	1·10
	796 ...	,, ,, ,, ...	·95
	798 ...	,, ,, ,, ...	·97
	147 Sample A	,, ,, breast	1·43
	,, B	,, ,, ,, ...	1·34
*	,, C	,, ,, ,, ...	1·20
	442 ,, A	,, ,, ,, ...	1·67
	,, B	,, ,, ,, ...	1·07
	789 ...	,, ,, ,, ...	·88
	*791 ...	,, ,, ,, ...	1·48
	795 ...	,, ,, ,, ...	1·06
	797 ...	,, ,, ,, ...	·98

* Tested for radium emanation.

A glance at the foregoing Table shows that no specimen of a normal tissue or of sarcoma afforded an acceleration of leak amounting to 10 per cent. of the natural leak; that 3 out of 8 (37·5 per cent.) secondary carcinomata showed a leak greater than 10 per cent. above the natural leak, the mean leak of all the 8 secondary carcinomata being 1·08 of the natural leak; and that 12 of the 27 samples (44·4 per cent.) of primary carcinoma showed a leak greater than 10 per cent. above the natural leak, while the mean leak of all these samples (omitting No. 697C because of its disturbing influence) was 1·21 of the natural leak.

In order to test the question further, and to determine whether the accelerated leak were due to the presence of a radio-active substance—in particular radium—in the carcinoma powders, substances were specifically examined for the presence of radium by means of the emanation electroscope.

The preliminary treatment of the substance was different in different cases, as will appear later; but the main treatment consisted in boiling the substance with radium-free hydrochloric acid in a specially-cleaned distilling flask in order to convert any radium into the chloride which is soluble, and to drive off any occluded radium emanation, sealing off the neck and side-piece of the flask, and setting aside the flask for four weeks in order to allow any radium to "grow" its emanation to the equilibrium value. At the end of four weeks the gas in the fluid was transferred by vigorous boiling into a reservoir, and thence, after passing over calcium chloride, into the metal reservoir of the emanation electroscope, where its leak was taken.

Samples of all the reagents used were examined by the emanation electroscope for the presence of radium, and the greatest care was taken to avoid contamination of any of the substances investigated with radium.

In all, 25 samples of different tissues were examined by the emanation method. From those contained in Table I. 7 were selected, and these were boiled with HCl, after their extraction with acetone; 18 other specimens were incinerated in an iron crucible either after extraction with ether or with acetone or fresh. In three instances the acetone extract was evaporated to dryness and incinerated as well as the residue.

TABLE II.

Reference No.	Substance.	Weight in Grams. Dry.	Weight in Grams. Fresh.	Preliminary Treatment.
437	Normal liver ...	2·2616	—	Acetone extraction ; 10 cc. HCl
833	„ „ ...	—	100	Incinerated ; 20 gm. deflagration mixture : 30 cc. HCl
836	„ „ ...	—	100	Incinerated : 20 gm. deflagration mixture ; 30 cc. HCl
440	Glands secondary to breast	2·321	—	Acetone extraction ; 10 cc. HCl
697C	Primary, cervix ...	1·00	—	Acetone extraction ; 10 cc. HCl
697G	„ „ ...	3·6124	—	Ether extraction ; 10 cc. HCl
793	Primary, tongue...	·2972	—	Acetone extraction ; 10 cc. HCl
147C	„ breast ...	3·7672	—	Ether extraction ; 10 cc. HCl
791	„ „ ...	2·3064	—	Acetone extraction ; 10 cc. HCl
806	„ liver ...	—	520	Acetone extraction ; residue incinerated to white ash; 10 cc. HCl
818	Acetone from 806	—	900 cc.	Evaporated ; incinerated to white ash ; 10 cc. HCl
807	Secondary in liver of 806	—	332 gm.	Acetone extraction ; incinerated to white ash ; 10 cc. HCl
819	Acetone from 807	—	770 cc.	Evaporated ; incinerated to white ash ; 10 cc. HCl
808	Hepatic substance from Case 806	—	332 gm.	Acetone extraction; incinerated to white ash ; 10 cc. HCl
820	Acetone from 808	—	910 cc.	Evaporated ; incinerated to white ash ; 10 cc. HCl
834	Primary, rectum...	—	44 gm.	Incinerated : 10 gm. deflagration mixture ; 15 cc. HCl
835	Intestine above and below growth 834	—	24 gm.	Incinerated ; 5 gm. deflagration mixture ; 7·5 cc. HCl
750	Primary, liver ...	5·072	—	Ether extraction : incineration ; 10 gm. deflagration mixture ; 15 cc. HCl
749	Hepatic substance from Case 750	4·405	—	Ether extraction ; incineration ; 10 gm. deflagration mixture ; 15 cc. HCl
751	Secondary in lung of Case 750	4·334	—	Ether extraction ; incineration ; 10 gm. deflagration mixture : 15 cc. HCl
746	Pulmonary substance of Case 750	11·27	—	Ether extraction ; incineration ; 10 gm. deflagration mixture ; 15 cc. HCl
837	Primary, stomach	—	24·5	Incinerated ; 5 gm. deflagration mixture ; 7·5 cc. HCl
838	Stomach around Growth 837	—	24·5	Incinerated ; 5 gm. deflagration mixture ; 7·5 cc. HCl
839	Primary, mediastinum	—	75·0	Incinerated : 15 gm. deflagration mixture ; 22·5 cc. HCl
844	Primary, splenic flexure	—	143·0	Incinerated : 15 gm. deflagration mixture : 22·5 cc. HCl
845	Intestine above and below Growth 844	—	143·0	Incinerated : 15 gm. deflagration mixture ; 22·5 cc. HCl
847	Primary, liver ...	—	164·0	Incinerated ; 30 gm. deflagration mixture ; 45 cc. HCl
848	Hepatic substance from Case 847	—	164·0	Incinerated ; 30 gm. deflagration mixture ; 40 cc. HCl

In most cases, after incineration of the substance to a black mass, this was ground to a fine powder and deflagrated with the addition of a weighed quantity of a mixture consisting of $NaNO_3$ (2 parts) Na_2CO_3 (1 part). The resulting mass was taken up with water and rendered acid with a known quantity of radium-free HCl.

Table II. gives a list of the 25 substances and 3 acetone extracts, with their preliminary treatment.

Table III. gives in tabular form the results of examining these substances for radium by the emanation electroscope. For purposes of comparison all results are given as estimated

TABLE III.

	Ref. No.	Substance.	Total Ra in Sample (net).	Ra per gram of Substance.
Normal.	437*	Normal liver	0	0
	833	,, ...	$2{\cdot}408 \times 10^{-8}$ mgr.	$2{\cdot}408 \times 10^{-10}$ mgr.
	836	,, ...	0	0
Non-malignant tissues from carcinoma cases.	808	Hepatic substance, case of primary carcinoma liver	$9{\cdot}7 \times 10^{-10}$ mgr.	$1{\cdot}08 \times 10^{-9}$ mgr.
	820		$3{\cdot}5734 \times 10^{-7}$ mgr.	
	749*	,, ,, ,,	0	0
	848	,, ,, ,,	$4{\cdot}108 \times 10^{-8}$ mgr.	$2{\cdot}5 \times 10^{-10}$ mgr.
	746*	Pulmonary substance, case of primary carcinoma liver	0	0
	835	Intestine, above and below primary growth...	$1{\cdot}37 \times 10^{-9}$ mgr.	$5{\cdot}7 \times 10^{-11}$ mgr.
	845	,, ,, ,,	0	0
	838	Stomach around primary growth	0	0
Secondary growths.	440	Lymphatic gland secondary to breast ...	$2{\cdot}37 \times 10^{-8}$ mgr.	$1{\cdot}08 \times 10^{-8}$ mgr.
	807	Liver growth secondary to primary carcinoma of liver	$2{\cdot}6 \times 10^{-9}$ mgr.	$4{\cdot}3 \times 10^{-10}$ mgr.
	819		$1{\cdot}396 \times 10^{-7}$ mgr.	
	751*	Pulmonary growth secondary to liver ...	0	0
Primary growths.	791*	Primary carcinoma, breast	$5{\cdot}63 \times 10^{-9}$ mgr.	$1{\cdot}88 \times 10^{-9}$ mgr.
	147C*	,, ,, ,,	0	0
	793*	,, ,, tongue	$1{\cdot}13 \times 10^{-7}$ mgr.	$3{\cdot}94 \times 10^{-7}$ mgr.
	697C*	,, ,, cervix	0	0
	697C**	,, ,, ,,	$1{\cdot}49 \times 10^{-5}$ mgr.	$2{\cdot}73 \times 10^{-6}$ mgr.
	806	,, ,, liver	$1{\cdot}25 \times 10^{-8}$ mgr.	$9{\cdot}1 \times 10^{-10}$ mgr.
	818		$4{\cdot}615 \times 10^{-7}$ mgr.	
	750*	,, ,, ,,	0	0
	847	,, ,, ,,	$3{\cdot}22 \times 10^{-9}$ mgr.	$2{\cdot}0 \times 10^{-11}$ mgr.
	834	,, ,, intestine	$9{\cdot}4 \times 10^{-10}$ mgr.	$2{\cdot}1 \times 10^{-11}$ mgr.
	844	,, ,, ,,	0	0
	837	,, ,, stomach	0	0
	839	,, ,, mediastinum	0	0

* Residue after extraction with acetone or with ether.

per gram of substance taken. In the case of those substances marked with an asterisk the estimate was made on the weight of dried extracted substance; in all other cases on the weight of the fresh substance. Full information concerning the results of individual experiments are given in the protocols at the end of the paper.

Partly because the amount of radium found was estimated on dried substances in some cases and on fresh in others, and partly because the actual amounts found differ so widely, consideration of mean values for the different groups is worthless. The most that can be said is that of 10 samples of non-malignant tissue, 4 showed the presence of radium, while of 15 samples of malignant tissue (primary and secondary) 8 showed the presence of radium.

Taking those substances in which non-malignant and malignant tissues were obtained from the same body we have—

I.	808 and 820	Hepatic substance	$1{\cdot}08 \times 10^{-9}$ mgr. per gram
	806 and 818	Primary carcinoma liver ...	$9{\cdot}1 \times 10^{-10}$ „
	807 and 819	Secondary carcinoma liver ...	$4{\cdot}3 \times 10^{-10}$ „
II.	749	Hepatic substance	0
	750	Primary carcinoma liver ...	0
	746	Pulmonary substance ...	0
	751	Secondary carcinoma lung ...	0
III.	848	Hepatic substance	$2{\cdot}5 \times 10^{-10}$ mgr. per gram
	847	Primary carcinoma liver ...	$2{\cdot}0 \times 10^{-11}$ „
IV.	835	Intestine	$5{\cdot}7 \times 10^{-11}$ „
	834	Primary carcinoma intestine	$2{\cdot}1 \times 10^{-11}$ „
V.	845	Intestine	0
	844	Primary carcinoma intestine	0
VI.	838	Stomach	0
	837	Primary carcinoma stomach	0

In reference to the above cases, however, it must be noted that it is only in the case of the liver and pancreas that strict comparison between the primary new growth and the type of cells from which that new growth arose can be made. To compare a columnar-cell carcinoma of intestine with intestine, though from the same body, is hardly fair, and in the case of the breast it is impossible to obtain normal glandular epithelium with which to compare the cancerous epithelium in respect of radium. It has appeared, further, that extraction with acetone removes part of any radium present, and this method has therefore been abandoned. Strict comparison is

only possible in Cases I. and III. above, and from them nothing can be deduced with safety as to the relative amounts of radium held by a cancerous epithelium and the normal epithelium from which it arises.

It is noteworthy, however, that if the twelve specimens of malignant and non-malignant tissues which have been found to contain radium be placed in order of magnitude of the contained radium, the first five places are taken by carcinomata.

Conclusion.

As a result of examining twenty-five samples of non-malignant tissues, non-malignant tissues from cases of carcinoma, secondary carcinomata, and primary carcinomata for radium by means of the emanation electroscope, small quantities of radium have been found in each group. Radium appears to be found somewhat more frequently and in larger, though still minute, quantity in carcinomatous than in non-carcinomatous tissue; but the point is not yet certain, since in three instances in which carcinomatous and non-carcinomatous tissues were obtained from the same body and in which radium was found, it was present in larger quantity in the non-carcinomatous tissue.

PROTOCOLS OF EXPERIMENTS.

I.—Stock Reagents.

January 17th, 1912.

10 cc. stock hydrochloric acid.

11.10 a.m. Natural leak 70th–60th division on scale 32′ 44″ = ·305 div./min.
12.22 p.m. Repeated 70th 49th „ „ 71′ 41″ = ·293 „
2.19–2.43 p.m. Boiled flask and let gas into reservoir of emanation electroscope.
2.48 p.m. Leak taken 70th–55th division on scale, 67′ 51″ = ·221 div./min.
3.57 p.m. Repeated 70th–62nd „ „ 31′ 15″ = ·256 „
No evidence of radium.

May 15th, 1912.

10 gm. of stock deflagrating mixture ($NaNO_3$ 2 parts + Na_2CO_3 1 part) fused in iron crucible, dissolved in water, 15 cc. stock HCl gradually added.

11.59 a.m. Natural leak 70th–65th division on scale 63′ 28″ = ·079 div./min.
1. 3 1.18 p.m. Flask boiled and gas let into reservoir of electroscope.
1.21 p.m. Leak taken 70th–67th division on scale 33′ 12″
1.59 p.m. Repeated 70th 65th „ „ 57′ 54″
3. 0 p.m. Repeated „ „ „ 54′ 1″ } = ·087 div./min.
3.55 p.m. Repeated „ „ „ 61′ 29″ }

Leak due to substance ·008 div./min., which corrected to radium standard indicates Ra = $8{\cdot}6 \times 10^{-10}$ mgr.

April 16th, 1912.

100 cc. stock acetone evaporated to dryness + 10 cc. stock HCl.

10.20 a.m. Natural leak 70th–65th division on scale 37′ 13″ = ·134 div./min.
11.0 a.m. Repeated „ „ „ 48′ 46″ = ·102 „
11.55 a.m.–12.8 p.m. Boiled flask and ran gas into reservoir of electroscope.
12.12 p.m. Leak taken 70th–65th division on scale 31′ 49″
12.48 p.m. Repeated „ „ „ 35′ 0″
1.27 p.m. Repeated „ „ „ 31′ 21″
3. 2 p.m. Repeated „ „ „ 32′ 14″
} = ·153 div./min.
Exhausted electroscope once.
3.58 p.m. Natural leak 70th–65th division on scale 42′ 31″
4.41 p.m. Repeated „ „ „ 42′ 41″
} = ·117 „

Leak due to substance = ·036 div./min., which estimated against Ra standard indicates $3{\cdot}49 \times 10^{-9}$ mgr. Ra.

April 24th, 1912.

Radium standard containing $1{\cdot}57 \times 10^{-7}$ mgr. Ra.

Natural leak 70th–60th division on scale, 19′ 6·6″ = ·523 div./min.
11.50 a.m.–12.4 p.m. Flask boiled and gas let into reservoir of electroscope.
12.13 p.m. Leak taken 70th–60th division on scale 6′ 24″
12.25 p.m. „ „ 6′ 20·8″ = 1·576 div./min.
12.58 p.m. „ „ 5′ 50·4″
1.28 p.m. „ „ 5′ 12·4″
2.37 p.m. „ „ 4′ 47·6″
3.11 p.m. „ „ 4′ 39·6″ = 2·143 div./min.
3.43 p.m. „ „ 5′ 1″
Electroscope exhausted and re-filled with dry air twice.
4.23 p.m. Natural leak 70th–60th division on scale 17′ 1·6″
4.44 p.m. „ „ „ 20′ 4″
5.43 p.m. „ 69th–59th „ 31′ 43″

Leak due to substance = { 1·62 divisions per minute at end of 3 hours.
1·053 „ „ 20 minutes.

II.—Tissues from Non-malignant Cases.

January 17th, 1912.

No. 437.—Normal human liver, acetone extracted and powdered 2·2616 gm. + 10 cc. stock HCl.

2.48 p.m. Natural leak 70th–55th division on scale 67′ 51″
3.57 p.m. „ 70th–62nd „ 31′ 15″
} = ·232 div./min.
4. 0–5.5 p.m. Flask boiled and gas let into reservoir of electroscope.
5. 8 p.m. Leak taken 70th–63rd division on scale 31′ 8″
5.40 p.m. Repeated 70th–56th „ 65′ 51·4′
} = ·216 div./min

No evidence of radium.

May 3rd, 1912.

No. 833.—100 gm. fresh normal human liver incinerated in iron crucible; 20 gm. stock deflagrating mixture; 30 cc. stock HCl.

12.50 p.m. Natural leak 70th–65th division on scale 61′ 7″ = ·082 div./min.
2.47–2.57 p.m. Boiled flask and ran gas into reservoir of electroscope.
2.58 p.m. Leak taken 70th–65th division on scale 22′ 53″ = ·218 div./min.
3.23 p.m. Repeated „ „ 20′ 47″ = ·241 „
3.46 p.m. Repeated „ „ 18′ 57″ = ·264 „
5.25 p.m. Repeated „ „ 16′ 56″ = ·295 „

Estimated on 9th day after sealing; corrected amount of Ra by Ra standard = $2{\cdot}58 \times 10^{-8}$ mgr. less $1{\cdot}72 \times 10^{-9}$ mgr. for deflagration mixture = $2{\cdot}408 \times 10^{-8}$ mgr. in 100 gm.

May 9th, 1912.

No. 836.—100 gm. fresh normal liver incinerated in iron crucible; 20 gm. stock deflagrating mixture; 30 cc. stock HCl.

10.15 a.m. Natural leak 70th–65th division on scale 65′ 21″
11.22 a.m. „ „ „ 70′ 4″ } = ·074 div./min.
12.30–12.45 p.m. Boiled flask and let gas into reservoir of electroscope.
12.45 p.m. Leak taken 70th–64th division on scale 78′ 64″ = ·076 div./min.
No sufficient evidence of radium.

III.—Non-Malignant Tissues from Malignant Cases.

February 27th, 1912.

No. 808.—332 gm. of fresh non-malignant liver from a case of primary malignant disease of the liver (No. 806); stock acetone extracted (see No. 820), incinerated to white ash without deflagrating mixture; 10 cc. stock HCl.

3.59 p.m. Natural leak 70th–65th division on scale 33′ 20″ = ·150 div./min.
4.44–5.4 p.m. Boiled flask and let gas into reservoir of electroscope.
5. 5 p.m. Leak taken 70th–65th division on scale 29′ 0″
5.36 p.m. Repeated „ „ 32′ 5″
6.10 p.m. Repeated „ „ 31′ 8·6″
6.45 p.m. Repeated „ „ 33′ 10.4″ } = ·160 div./min.
7.21 p.m. Repeated „ „ 32′ 23″
7.56 p.m. Repeated „ „ 30′ 6·6″
Electroscope exhausted twice.
8.59 p.m. Natural leak 70th–65th division on scale 33′ 3·8″
9.34 p.m. Repeated „ „ 32′ 8·4″
10.25 p.m. Repeated „ „ 33′ 31″ = ·150 div./min.
11. 2 p.m. Repeated „ „ 35′ 13″

Leak due to substance = ·01 div./min., which estimated against the Ra standard = $9{\cdot}7 \times 10^{-10}$ mgr. Ra.

April 16th, 1912.

No. 820.—910 cc. stock acetone extract of liver of which No. 808 was residue. Evaporated to dryness, incinerated to white ash; 10 cc. stock HCl.

3.58 p.m.	Natural leak 70th–65th division on scale 42′ 31″	= ·117 div./min.
4.41 p.m.	Repeated „ „ „ 42′ 41″	
5.25–5.43 p.m.	Boiled flask and let gas into reservoir of electroscope.	
5.43 p.m.	Leak taken 70th–65th division on scale 1′ 56″	
5.47 p.m.	Repeated „ „ „ 1′ 56·2″	
5.53 p.m.	Repeated „ „ „ 1′ 50·2″	= 2·727 div./min.
6. 4 p.m.	Repeated „ „ „ 1′ 49·2″	
6.10 p.m.	Repeated „ „ „ 1′ 40·2″	
	Electroscope exhausted twice.	
7. 7 p.m.	Natural leak 70th–65th division on scale 8′ 20·4″	
8.29 p.m.	Repeated „ „ „ 23′ 40″	
8.55 p.m.	Repeated „ „ „ 37′ 52″	

Leak due to substance at end of 20 minutes in electroscope = 2·61 div./min., which, corrected by Ra standard (giving 1·053 div./min. at end of 20 minutes), indicates $3{\cdot}891 \times 10^{-7}$ mgr. Ra *less* $\frac{9}{100} \times 3{\cdot}49 \times 10^{-9}$ mgr. for acetone = $3{\cdot}5734 \times 10^{-7}$ mgr. Ra.

May 8th, 1912.

No. 749.—4·405 gm. of dry ether-extracted liver; incinerated; 10 gm. stock deflagrating mixture; 15 cc. stock HCl.

11.35 a.m.	Natural leak 70th–63rd division on scale 77′ 0″ = ·091 div./min.	
12.58–1.9 p.m.	Flask boiled and gas let into electroscope.	
1.19 p.m.	Leak taken 70th–65th division on scale 51′ 34″	= ·090 div./min.
2.12 p.m.	Repeated „ „ „ 59′ 9″	
	No evidence of radium.	

June 8th, 1912.

No. 848.—164 gm. of fresh non-malignant liver from a case of primary carcinoma of the liver surrounding the gall bladder. Incinerated; 30 gm. stock deflagrating mixture; 40 cc. stock HCl.

	Natural leak 70th–65th division on scale 68′ 2″ = ·073 div./min.	
11.14–11.30 a.m.	Boiled flask and let gas into reservoir of electroscope.	
11.32 a.m.	Leak taken 70th–65th division on scale 18′ 0″	= ·278 div./min.
11.51 a.m.	Repeated „ „ „ 18′ 8″	

Estimated on 7th day after sealing flask. Corrected to Ra standard at 20 minutes after letting gas into reservoir. Leak due to substance = ·205 div./min. Ra = $4{\cdot}366 \times 10^{-8}$ mgr. *less* $·258 \times 10^{-8}$ mgr. for deflagrating mixture or $4{\cdot}108 \times 10^{-8}$ mgr.

May 8th, 1912.

No. 746.—11·27 gm. dry ether-extracted pulmonary tissue from case of primary carcinoma of liver with metastases in lung. Incinerated; 20 gm. stock deflagration mixture; 30 cc. stock HCl.

11.35 a.m. Natural leak 70th–63rd division on scale 77′ 0″ = ·091 div./min.
5.55–6.5 p.m. Boiled flask and let gas into reservoir.
6. 5 p.m. Leak taken 70th–65th division on scale 54′ 10″ = ·092 div./min.
No evidence of radium.

May 7th, 1912.

No. 835.—24 gm. fresh intestine (mucous membrane and muscle) above and below growth No. 834. Incinerated; 5 gm. stock deflagrating mixture; 7·5 cc. stock HCl.

10. 3 a.m. Natural leak 70th–65th division on scale 71′ 36″
11.16 a.m. Repeated 70th–67th „ „ 32′ 54″ } = ·070 div./min.
3.14–3.26 p.m. Boiled flask and let gas into reservoir of electroscope.
3.27 p.m. Leak taken 70th–65th division on scale 59′ 43″ = ·084 div./min.
4.29 p.m. Repeated „ „ „ 62′ 4″ = ·081 „
Estimated on 11th day after sealing flask. Leak due to substance = ·012 div./min. = 1·8 × 10⁻⁹ mgr. Ra *loss* ·43 × 10⁻⁹ for deflagrating mixture = 1·37 × 10⁻⁹ mgr.

May 22nd, 1912.

No. 845.—143 gm. fresh intestine above and below growth No. 844. Incinerated; 15 gm. stock deflagrating mixture; 22·5 cc. stock HCl.

11.50 a.m. Natural leak 70th–65th division on scale, 54′ 8″ = ·092 div. min.
12.50–1.0 p.m. Boiled flask and let gas into reservoir of electroscope.
1. 3 p.m. Leak taken 70th–65th division on scale, 48′ 57″ = ·102 div./min.
1.56 p.m. Repeated „ „ 55′ 28″ = ·090 „
No evidence of radium.

May 9th, 1912.

No. 838.—24·5 gm. fresh stomach around growth No. 837. Incinerated: 5 gm. stock deflagration mixture; 7·5 cc. stock HCl.

10.15 a.m. Natural leak 70th–65th division on scale 65′ 21″
11.22 a.m. Repeated „ „ 70′ 4″ } ·074 div./min.
4.25–4.35 p.m. Boiled flask and let gas into reservoir of electroscope.
4.37 p.m. Leak taken 70th–65th division on scale, 66′ 15″ = ·075 div./min.
No evidence of radium.

IV.—Secondary Growths.

January 19th, 1912.

No. 440.—2·321 gm. dried acetone extracted growth from axillary glands secondary to breast. Boiled with 10 cc. HCl.

10.20 a.m. Natural leak 70th–67th division on scale 11′ 40″
10.40 a.m. Repeated 70th–64th „ 24′ 25″ ·244 div./min.
11. 9 a.m. Repeated 70th–59th „ 46′ 4″
11.57 a.m.–12.13 p.m. Boiled flask and let gas into reservoir of electroscope.
12.15 p.m. Leak taken 55th–30th division on scale 60′ 58″=·410 div./min.
1.17 p.m. „ 77th–44th „ 62′ 4″ =·532 „
3. 5 p.m. „ „ „ 68′ 15″=·484 „
Electroscope exhausted twice.
4.43 p.m. Natural leak 40th–30th division on scale 42′ 54″=·233 div./min.
5.30 p.m. Repeated 66th–54th „ 50′ 1″=·240 „

Leak due to substance = ·484 *less* ·239 (mean natural leak), which calculated on Ra standard = $2·37 \times 10^{-8}$ mgr. Ra.

February 27th, 1912.

No. 807.—332 gm. of fresh secondary hepatic metastasis in a case of primary carcinoma of liver (No. 806). Extracted stock acetone (No. 819). Incinerated to white ash: 10 cc. stock HCl.

9.25 a.m. Natural leak 70th–65th division on scale 29′ 56·4″ = ·167 div./min.
10. 7–10.29 a.m. Flask boiled and gas let into reservoir of electroscope.

			Leak div./min.	Estim'd N.L. div./min.	Leak due to substance div./min.
10.31 a.m.	Leak taken 70th–65th div. on scale	29′ 26″	= ·170	*less* ·161	=·0135
11. 3 a.m.	Repeated „ „	28′ 14·6″	= ·177	„ ·159	
11.34 a.m.	Repeated „ „	29′ 15·2″	= ·171	„ ·156	=·018
12. 5 p.m.	Repeated „ „	28′ 41·4″	= ·174	„ ·153	
12.36 p.m.	Repeated „ „	28′ 22·8″	= ·176	„ ·150	=·027
1. 7 p.m.	Repeated „ „	28′ 30·6″	= ·175	„ ·149	
	Electroscope exhausted twice.				
2. 9 p.m.	Natural leak 70th–65th div. on scale	29′ 10·2″	= ·171	„ ·141	=·030
2.41 p.m.	„ „ „	35′ 16″	= ·142	„ ·138	=·004
3.19 p.m.	„ „ „	37′ 22·4″	= ·134	„ ·134	= 0

Calculated by Ra standard = $2·6 \times 10^{-9}$ mgr. Ra.

March 20th, 1912.

No. 819.—770 cc. stock acetone extract of which No. 807 was dried residue. Incinerated to white ash; 10 cc. stock HCl.

9.45 a.m. Natural leak 70th–65th division on scale, 44′ 57″=·111 div. min.
10.32–10.50 a.m. Boiled flask and let gas into reservoir of electroscope.
10.52 a.m. Leak taken 70th–65th div. on scale 3′ 39″
10.58 a.m. „ „ „ 3′ 29″
11. 7 a.m. „ „ „ 3′ 43″
11.13 a.m. „ „ „ 3′ 21″ 3′ 22·4″=1·48 div./min.
11.19 a.m. „ „ „ 3′ 21″ for first hour.
11.23 a.m. „ „ „ 3′ 4″
11.35 a.m. „ „ „ 3′ 0″

12.15 p.m.	Leak taken 70th–65th div. on scale		2′ 50·2″	2′ 45·2″ = 1·82 div./min.
12.44 p.m.	„	„	„	2′ 40·2″	for second hour.
12.53 p.m.	„	„	„	2′ 30·4″	
12.58 p.m.	„	„	„	2′ 42·4″	
1. 4 p.m.	„	„	„	2′ 36″	
1.10 p.m.	„	„	„	2′ 34·4″	2′ 38·5″ = 1·893 div./min.
1.27 p.m.	„	„	„	2′ 43″	for third hour.
1.44 p.m.	„	„	„	2′ 44″	
1.51 p.m.	„	„	„	2′ 39·4″	

Electroscope exhausted twice.

3. 9 p.m. Natural leak 70th–65th division on scale 17′ 54·4″
4.21 p.m. „ „ „ 45′ 18″

Leak due to substance = 1·782 div./min., which, calculated on Ra standard = $1{\cdot}665 \times 10^{-7}$ *less* $\frac{1}{100} \times 3{\cdot}49 \times 10^{-9}$ ($= {\cdot}269 \times 10^{-7}$) for acetone. Hence No. 819 contains $1{\cdot}396 \times 10^{-7}$ mgr. Ra.

May 8th, 1912.

No. 751.—4·334 gm. of ether-extracted dry pulmonary metastasis from a case of primary carcinoma of liver (No. 750); incinerated; 10 gm. stock deflagrating mixture; 10 cc. stock HCl.

11.35 a.m. Natural leak 70th–63rd division on scale 77′ 0″ = ·091 div./min.
4.25–4.45 p.m. Boiled flask and let gas into reservoir of electroscope.
4.46 p.m. Leak taken 70th–65th division on scale 54′ 34″ = ·092 div./min.
No evidence of radium.

V.—Primary Malignant Growths (Carcinomata).

January 20th, 1912.

No. 791. — 2·3064 gm. acetone-extracted dry primary spheroidal-cell carcinoma of breast; 10 cc. stock HCl.

11.53 a.m. Natural leak 70th–59th division on scale 49′ 53″ = ·221 div./min.
12.45–1.1 p.m. Boiled flask and let gas into reservoir of electroscope.

			Leak div./min.	Estim'd N.L. div./min.	Leak due to substance div./min.
1. 4 p.m.	Leak taken 56th–46th division on scale	42′ 15″ =	·237	*less* ·216 =	·021
1.50 p.m.	Repeated 70th–50th	„ 74′ 20″ =	·269	„ ·211 =	·058
3.16 p.m.	Repeated 70th–56th	„ 53′ 10″ =	·263	„ ·204 =	·059

Electroscope exhausted twice.

4.43 p.m. Natural leak 50th–39th division on scale 58′ 34″ = ·188 div./min.

Leak due to substance calculated to Ra standard indicates $5{\cdot}63 \times 10^{-9}$ mgr. Ra.

January 18th, 1912.

No. 147C. — 3·7672 gm. dry ether-extracted primary spheroidal-cell carcinoma of breast; 10 cc. stock HCl.

11.18 a.m. Natural leak 70th–59th division on scale 54′ 55″ = ·200 div./min.
2.45–3.10 p.m. Boiled flask and let gas into reservoir of electroscope.
3.12 p.m. Leak taken 67th–52nd division on scale 76′ 20″ = ·196 div./min.
Electroscope exhausted twice.
Natural leak 52nd–40th division on scale 64′ 56″ = ·187 div./min.
No evidence of radium.

January 22nd, 1912.

No. 793.—·2972 gm. acetone-extracted dry squamous-cell carcinoma of tongue; 10 cc. stock HCl.

12. 0 noon. Natural leak 70th–56th division on scale 45′ 38″=·307 div./min.
12.50–1.15 p.m. Boiled flask and let gas into reservoir of electroscope.

1.16 p.m.	Leak taken	45th–40th	division on scale	4′ 4·6″
1.23 p.m.	Repeated	70th–50th	„	16′ 8″
1.42 p.m.	Repeated	„	„	15′ 16″
2. 2 p.m.	Repeated	„	„	14′ 32″
3. 5 p.m.	Repeated	„	„	11′ 57″
3.24 p.m.	Repeated	„	„	13′ 0″
3.39 p.m.	Repeated	„	„	13′ 8″
4. 1 p.m.	Repeated	„	„	13′ 32·4″ } =1·5 div./min.

Electroscope exhausted twice.

4.56 p.m. Natural leak, 70th–50th division on scale 48′ 10″
5.48 p.m. Repeated 70th–55th „ 50′ 14″=·299 div./min.

Leak due to substance = 1·2 div./min., which calculated to Ra standard = $1{\cdot}13 \times 10^{-7}$ mgr. Ra.

January 18th, 1912.

No. 697G. — 3·6124 gm. dry ether-extracted primary squamous-cell carcinoma of cervix; 10 cc. stock HCl.

11.18 a.m. Natural leak 70th–59th division on scale 54′ 55″=·200 div./min.
12.25–12.44 p.m. Boiled flask and let gas into reservoir of electroscope.
12.45 p.m. Leak taken 54th–38th division on scale 88′ 15·6″=·181 div./min.
No evidence of radium.

January 23rd, 1912.

No. 697C. — ·5458 gm. dry acetone-extracted primary squamous-cell carcinoma of cervix; 10 cc. stock HCl.

11.30 a.m. Natural leak 70th–60th division on scale 31′ 24″=·319 div./min.
12.3–12.26 p.m. Boiled flask and let gas into reservoir of electroscope.

12.26 p.m.	Leak taken	70th–30th	division on scale	21·4″
	Repeated	„	„	21·0″
	Repeated	„	„	20·8″
12.31 p.m.	Repeated	„	„	20·0″
12.39 p.m.	Repeated	„	„	19·6″
1. 6 p.m.	Repeated	„	„	17·6″
1.21 p.m.	Repeated	„	„	16·4″
2.45 p.m.	Repeated	„	„	15·6″
2.47 p.m.	Repeated	„	„	15·6″
3.32 p.m.	Repeated	„	„	15·6″=153·8 div./min.

Electroscope exhausted twice.

4.11 p.m.	Natural leak	70th–30th	division on scale	1′ 8″
4.53 p.m.	Repeated	„	„	2′ 23·6″
5.23 p.m.	Repeated	„	„	4′ 19·6″
6.10 p.m.	Repeated	„	„	12′ 3·6″

Leak due to substance calculated to Ra standard indicates $1{\cdot}49 \times 10^{-5}$ mgr. Ra.

February 26th, 1912.

No. 806. — 520 gm. fresh primary carcinoma of liver extracted stock acetone. Incinerated to white ash; 10 cc. stock HCl.

10.34 a.m. Natural leak 70th–65th division on scale 22′ 40″ = ·221 div./min.
11.8–11.26 a.m. Flask boiled and gas let into reservoir of electroscope.

			Leak div. min.	Estimated N.L. div. min.	Leak due to substance div./min.
11.27 a.m.	Leak taken 70th–65th div. on scale	15′ 52″	·315 *less* ·213		= ·093
11.45 a.m.	Repeated „ „	16′ 29″	·303 „ ·211		for
12. 8 p.m.	Repeated „ „	16′ 43·2″	·299 „ ·208		first
12.22 p.m.	Repeated „ „	17′ 8·4″	·292 „ ·204		hour.
12.41 p.m.	Repeated „ „	16′ 6·4″	·310 „ ·202		= ·115
1. 0 p.m.	Repeated „ „	15′ 44·6″	·318 „ ·200		for
1.17 p.m.	Repeated „ „	15′ 42·4″	·318 „ ·198		second
1.35 p.m.	Repeated „ „	missed.			hour.
1.53 p.m.	Repeated „ „	15′ 50″	·316 „ ·190		= ·129
2.14 p.m.	Repeated „ „	16′ 2″	·312 „ ·188		for
2.31 p.m.	Repeated „ „	15′ 29″	·323 „ ·183		third
2.50 p.m.	Repeated „ „	16′ 14″	·308 „ ·181		hour.
	Electroscope exhausted twice.				
3.38 p.m.	Natural leak 70th–65th div. on scale	24′ 12″	·207 „ ·173	= ·034	
4. 5 p.m.	Repeated „ „	28′ 11″	·177 „ ·169	= ·008	
4.35 p.m.	Repeated „ „	30′ 34″	·164 „ ·164	= · 0	
5. 8 p.m.	Repeated „ „	32′ 6″	·156 „ ·159	= ·003	
5.43 p.m.	Repeated „ „	31′ 39″	·158 „ ·157	= ·001	

Leak due to substance calculated to Ra standard indicates $1{\cdot}25 \times 10^{-}$ mgr. Ra.

March 18th, 1912.

No. 818. — 900 cc. stock acetone extract of No. 806. Incinerated to white ash; 10 cc. stock HCl.

10. 0 a.m. Natural leak 70th–65 division on scale 33′ 35″ } = ·150 div./min
10.46 a.m. Repeated „ „ „ 33′ 17″ }
11.22–11.36 a.m. Boiled flask and let gas into reservoir of electroscope.

11.37 a.m.	Leak taken 70th–65th division on scale	1′ 33·4″
11.41 a.m.	„ „ „	1′ 25·2″
11.46 a.m.	„ „ „	1′ 20·4″
11.48 a.m.	„ „ „	1′ 20·6″
11.54 a.m.	„ „ „	1′ 22·6″
12. 1 p.m.	„ „ „	1′ 18·4″
12. 6 p.m.	„ „ „	1′ 14·8″
12.10 p.m.	„ „ „	1′ 11″
12.14 p.m.	„ „ „	1′ 12″
12.17 p.m.	„ „ „	1′ 13·6″
12·25 p.m.	„ „ „	1′ 14″
12·40 p.m.	„ „ „	1′ 9·2″
1.14 p.m.	„ „ „	1′ 3·6″
1.20 p.m.	„ „ „	1′ 3·4″
1.47 p.m.	„ „ „	58″
1.49 p.m.	„ „ „	58·6″

2.21 p.m. Leak taken 70th–65th division on scale 1′ 2·4″
2.25 p.m. „ „ „ 58·8″
2.31 p.m. „ „ „ 56·4″
2.35 p.m. „ „ „ 59·2″ } = 5·236 div./min.
2.50 p.m. „ „ „ 56·4″
Electroscope exhausted twice.
3.56 p.m. Natural leak 70th–65th division on scale 5′ 33·6″
4. 7 p.m. „ „ „ 7′ 1·8″
4.48 p.m. „ „ „ 18′ 7″
5.35 p.m. „ „ „ 30′ 24″

Leak due to substance calculated to Ra standard indicates $4{\cdot}929 \times 10^{-7}$ mgr. Ra *less* $\frac{900}{100} \times 3{\cdot}49 \times 10^{-9}$ ($=\cdot 314 \times 10^{-7}$) for acetone, i.e. $4{\cdot}615 \times 10^{-7}$ mgr. Ra.

May 8th, 1912.

No. 750.—5·072 gm. ether-extracted primary carcinoma of liver. Incinerated; 10 gm. stock deflagrating mixture; 15 cc. stock HCl.

10.35. a.m. Natural leak 70th–63rd division on scale 77′ 0″=·091 div./min.
3.13–3.30 p.m. Boiled flask and let gas into reservoir of electroscope.
3.31 p.m. Leak taken 70th–65th division on scale, 53′ 36″=·093 div./min.
No evidence of radium.

June 6th, 1912.

No. 847.—164 gm. fresh primary carcinoma of liver encircling but not destroying gall bladder. Incinerated; 30 gm. stock deflagrating mixture; 45 cc. stock HCl. Estimated on sixth day after sealing flask.

12.40 p.m. Natural leak 70th–65th division on scale, 50′ 38″=·099 div./min.
1.31–1.54 p.m. Flask boiled and gas let into reservoir of electroscope.
2.10 p.m. Leak taken 70th–62nd division on scale 67′ 35″=·118 div./min.
3.20 p.m. Repeated 70th–65th „ 49′ 53″=·135 „
5.47 p.m. Repeated 70th–68th „ 21′ 43″=·138 „

Leak due to substance calculated and corrected to Ra standard indicates $5{\cdot}8 \times 10^{-9}$ mgr. Ra *less* $2{\cdot}58 \times 10^{-9}$ mgr. for deflagrating mixture, i.e. $3{\cdot}22 \times 10^{-9}$ mgr. Ra.

May 7th, 1912.

No. 834. — 44 gm. fresh primary carcinoma of rectum. Incinerated; 10 gm. stock deflagrating mixture; 10 cc. stock HCl. Estimated on eleventh day after sealing flask.

10. 3 a.m. Natural leak 70th–65th division on scale 71′ 36″=·070 div./min.
11.49 a.m.–12.6 p.m. Boiled flask and let gas into reservoir of electroscope.
12. 7 p.m. Leak taken 70th–65th division on scale 59′ 38″=·083 div./min.
1.12 p.m. Repeated 70th–62nd „ 97′ 54″=·082 „

Leak due to substance calculated and corrected to Ra standard indicates $1{\cdot}8 \times 10^{-9}$ mgr. Ra *less* $8{\cdot}6 \times 10^{-10}$ mgr. for deflagrating mixture; i.e. $9{\cdot}4 \times 10^{-10}$ mgr. Ra.

May 22nd, 1912.

No. 844.—143 gm. fresh primary carcinoma of splenic flexure. Incinerated; 15 gm. stock deflagrating mixture; 22·5 cc. stock HCl. Estimated on sixth day after sealing flask.

11.50 a.m. Natural leak 70th–65th division on scale 54′ 8″=·092 div./min.
2.52–3.8 p.m. Boiled flask and let gas into reservoir of electroscope.
3. 9 p.m. Leak taken 70th–63rd division on scale 79′ 7″=·088 div./min.
4.29 p.m. Repeated 70th–65th ,, 48′ 17″=·103 ,,

Leak due to substance calculated and corrected to Ra standard=6×10^{-10} mgr. Ra *less* $12{\cdot}9 \times 10^{-10}$ mgr. due to deflagrating mixture; i.e. no evidence of radium.

May 9th, 1912.

No. 837.—24·5 gm. fresh primary carcinoma of stomach. Incinerated; 5 gm. stock deflagrating mixture; 7·5 cc. stock HCl.

10.15 a.m. Natural leak 70th–65th division on scale 65′ 21″
11.22 a.m. Repeated ,, ,, 70′ 4″ } =·074 div./min.
3.8–3.25 p.m. Boiled flask and let gas into reservoir of electroscope.
3.29 p.m. Leak taken 70th–65th division on scale 68′ 23″=·073 div./min.
No evidence of radium.

May 10th, 1912.

No. 839.—75 gm. fresh malignant growth (? columnar-cell carcinoma) in mediastinum. Incinerated; 15 gm. stock deflagrating mixture; 22·5 cc. stock HCl.

1. 1 p.m. Natural leak 70th–65th division on scale 51′ 6″=·098 div./min.
1.55–2.16 p.m. Boiled flask and let gas into reservoir of electroscope.
2.17 p.m. Leak taken 70th–65th division on scale 55′ 16″=·091 div./min.
3.16 p.m. Repeated 70th–66th ,, 43′ 44″=·091 ,,
Electroscope exhausted once.
4.19 p.m. Natural leak 70th–61st division on scale 112′ 46″=·080 div./min.
No evidence of radium.

ON THE PRESENCE OF RADIUM IN SOME GALLSTONES AND ON A CORRELATION OF THIS WITH THE FREQUENCY OF GALLSTONE OCCURRENCE IN CARCINOMA.

By W. S. LAZARUS-BARLOW.

For the purposes of the present research samples of gallstones derived from 15 cases* have been examined. Of these, 7 individuals died from causes other than malignant disease, 5 died from carcinoma in some part of the body other than the gall-bladder, and 3 died of malignant disease primarily affecting the gall-bladder.

The gallstones have in all instances been examined as to the presence of radium by means of the emanation electroscope but the preliminary treatment for obtaining solution has been of two kinds: 1° removal of the cholesterin by radium-free ether and boiling of the residue with 10 cc. radium-free hydrochloric acid in water; 2° incineration of the gallstone in a platinum dish and solution of the residue in 1 cc. radium-free hydrochloric acid in water. The first method has the advantage that at no stage is the temperature raised above 100° C., but the disadvantages that the procedure is more complicated and that solution is not quite complete; the second method has the advantages that solution is complete and that the procedure is simple, but disadvantages that the temperature required is higher and that in process of incineration sooty particles derived from the cholesterin are given off in large quantities and may carry radium along with them. In eight instances calculi were examined by both methods.

It must here be mentioned that it is impossible to be certain—though grinding of a material be carried out till the powder is very fine—that samples derived from the same stock

* Two other cases have been examined since writing this paper; these are referred to in the note on p. 115, and the protocols of the experiments are given at the end of the paper.

will contain the same amounts of radium. This was shown by examination of several samples of a stock powder derived from a single specimen of carcinoma. In a paper by the author communicated to the Royal Society on March 14th, 1912,* examples were given which indicate that successive samples from a single growth may vary within very wide limits as concerns their radium content. Hence certainty as to the presence or absence of radium, and in particular as to the amount of radium, can only be satisfactorily arrived at if the entire specimen (whether mass of carcinoma or collection of gallstones) in a given case be dealt with. Such a course has manifest disadvantages, but it is clear that in cases where it occurs radium is disposed in "pockets," or else, being present only in minute quantity, is unevenly distributed throughout a powder. Strictly speaking, therefore, examination of different samples of a single stock by different methods affords no evidence of the superiority of one method over the other.

After preliminary preparation of amounts varying from ·8 to 6·33 gm. of gallstone, by one or other of the above methods, the hydrochloric acid solutions were transferred to flasks with side piece, boiled thoroughly, sealed off in the flame, set aside for four weeks, and at the end of that time examined for emanation.

Throughout the entire series of manipulations the greatest care was taken to avoid contamination. Stock solutions of distilled water, hydrochloric acid, and ether were used, samples of which were found to be radium-free by the emanation method. All utensils were kept separate, and well washed prior to use with some of the above hydrochloric acid and water.

In the case of doubtful presence of radium, i.e. when the leak on introduction of the boiled-off gas into the electroscope differed but little from the natural leak, determination of rate of leak was continued till the end of the third hour, and the presence (and amount) of radium was only concluded if there occurred a steady increase in leak hour by hour, and also if the leak after twice exhausting the electroscope was

* "On the Presence of Radium in some Carcinomatous Tumours" "Proc. Roy. Soc. 1912," B. vol. 85. Cf. also this vol., p. 91.

less than that obtaining prior to exhaustion and showed a progressive diminution until it equalled the natural leak at the commencement of the experiment, from decay of active deposit. In those instances in which a relatively considerable amount of emanation was indicated by the first observation taken after introducing the gas into the electroscope, the amount of radium was estimated on the values obtained 20 minutes after running the gas in, i.e. at a time when the first level period was attained.

The results obtained are set forth in Tables I., II., and III., from which it appears that of the 8 specimens examined by both methods, 3 specimens afforded evidence of the presence of radium by both methods, in 3 specimens no radium was found by either method, and in 2 specimens radium was found by the ether-extraction method but not by the incineration method. Of the remaining 7 specimens, which were completely used for radium estimation, and which, therefore, could only be examined by a single method, 5 afforded more or less conclusive evidence of the presence of radium, in 2 it was not found. Taken together, 5 specimens of gallstones afforded no evidence of the presence of radium, while 10 specimens showed the presence of radium.

Consideration of the amounts of radium found in various specimens is deferred to a later paragraph. The actual amounts present were estimated by comparison with a standard Ra solution containing $1{\cdot}57 \times 10^{-7}$ mgr. Ra.

A Possible Association of Radium in Gallstones with Bacteria.

While there can be no doubt that the radium which ultimately is found in the gallstones arrives in the body generally from outside, the mechanism whereby the radium becomes located in the gallstone is unknown. It is commonly held that the nucleus around which cholesterin, bile pigment, salts are deposited in the formation of a gallstone, is of bacterial nature. If this be so, the following experiment indicates a possible explanation for the presence of radium in gallstones. A standard nutrient broth was made, containing a known amount of radium. In it were grown bacteria of different kinds, and when copious growth had been obtained

TABLE I.—Radium Estimations in Gallstones. Ether-extraction Method.

Reference Number and Disease.	Natural Leak.		Experimental Leak *less* Natural Leak. (Leak due to Substance.)						Total Ra found.	Ra per gm. of Sample tested.	Ratio.
	Initial div./min.	Final div./min.	1st hour. div./min.	2nd hour. div./min.	3rd hour. div./min.		1st hour. div./min.	2nd hour. div./min.	3rd hour div./min.	$\times 10^{-10}$ mgr.	$\times 10^{-10}$ mgr.	
799. Carcinoma of gall-bladder	·220	·142	·401	·466	·476		·131	·045	·017	475	594·0	24·7
809. Carcinoma of gall-bladder	·210	·144	·877	1·076	1·135		·187	·030	·004	1100	220·0	
810. Carcinoma of gall-bladder	·102	·101	·111	·141	·163		·037	·020	0	158	129·0	
801. Carcinoma of breast ...	·188	·158	·149	·178	·191		·042	·014	0	185	181·4	3·8
802. Carcinoma of urinary bladder	·188 ·096	·182 ·118	+·013 −·003	−·021 +·010	+·007 +·011		— +·017	— −·001	— +·032	None.	None.	
803. Carcinoma of vulva ...	·176	·174	−·008	−·038	−·008		—	—	—	None.	None.	
804. Carcinoma of rectum ...	·159	·152	·058	·073	·078		·015	·003	0	80·4	35·9	
811. Carcinoma of stomach ...	·097	·097	·030	·031	·032		·011	0	·009	31·0	22·1	
805. Non-malignant	·164	·144	·0002	·0102	·0099		·0149	−·0039	+·0021	None.	None.	1
809. Non-malignant	·103	·096	·055	·069	·077		·025	·014	0	74·6	31·2	
812. Non-malignant	·109	·093	·020	·033	·035		·024	·003	0	33·9	10·1	
813. Non-malignant	·095	·098	·020	·010	·004		·021	·003	0	None.	None.	
821. Non-malignant	·106	·099	·029	·032	·032		·012	·001	0	31·0	29·1	
822. Non-malignant	·100	·092	·022	·031	·025		·019	0	0	27·1	5·8	

Electroscope exhausted twice.

Note.—In 6·33 gm. of the cholesterin contained in the mixed ether extract of all the above calculi no radium was found. (N.L.=·0732 div./min. Exp. leak = ·0714 div./min.)

TABLE II.

RADIUM ESTIMATIONS IN GALLSTONES.

INCINERATION METHOD.

Reference Number and Disease.	Natural Leak. div./min.	Experimental Leak. div./min.	Leak due to the Substance. div./min.	Radium found per gram of Gall-stone. $\times 10^{-10}$ mgr.
799. Carcinoma of gall-bladder ...	·110	1·552	1·442	717
800. Carcinoma of gall-bladder ...	·0807	·9063	·8256	410
801. Carcinoma of breast	·100	·095	—·005	None
802. Carcinoma of urinary bladder	·113	·105	—·008	None
803. Carcinoma of vulva	·110	·097	—·003	None
804. Carcinoma of rectum ...	·097	·112	·015	6·2
805. Non-malignant	·107	·101	—·006	None
809. Non-malignant	·100	·096	—·004	None
829. Non-malignant	·106	·100	—·006	None

TABLE III.

RADIUM ESTIMATIONS IN GALLSTONES.

Comparisons of amounts found by ether-extraction and incineration methods. From Tables I. and II. Per gram of gallstone.

Reference Number and Disease.	Ether-extraction method.	Incineration method.
799. Carcinoma of gall-bladder ...	594 $\times 10^{-10}$ mgr.	717 $\times 10^{-10}$ mgr.
800. Carcinoma of gall-bladder ...	220 ,,	410 ,,
810. Carcinoma of gall-bladder ...	129 ,,	—
801. Carcinoma of breast	181·4 ,,	None
802. Carcinoma of urinary bladder...	None	None
803. Carcinoma of vulva	None	None
804. Carcinoma of rectum	35·9 ,,	6·2 $\times 10^{-10}$ mgr.
811. Carcinoma of stomach	22·1 ,,	—
805. Non-malignant	None	None
809. Non-malignant	31·2 ,,	None
812. Non-malignant	10·1 ,,	—
813. Non-malignant	None	—
821. Non-malignant	29·1 ,,	—
822. Non-malignant	5·8 ,,	—
829. Non-malignant	—	None

the bacteria were separated by centrifugalisation. Deposit and supernatant broth were then separately taken in known quantities, stock hydrochloric acid added, boiled in a flask with side piece, set aside for four weeks, and examined by the emanation method for the amounts of radium they severally contained. Detailed explanation of the experiments is not

needed, since events showed that the bacterial deposit contained amounts of radium too great for accurate estimation by the emanation method. Clear evidence, however, is afforded that bacteria, whether living or dead—whether in pure culture or not—whether grown at 37° C. or at room temperature, accumulate to themselves radium from a radium-containing fluid in which they are placed.

The following is a short account of the results:—

(1) As estimated by the emanation electroscope, the original "radium broth" contained $1{\cdot}74 \times 10^{-5}$ mgr. Ra per cc. After *killed Staphylococcus pyogenes aureus* had remained in 300 cc. broth for 12 days at 37° C. the centrifuged deposit (·15 cc.) contained radium at the rate of $4{\cdot}3 \times 10^{-3}$ mgr. per cc.; the supernatant broth corrected for evaporation contained $1{\cdot}003 \times 10^{-5}$ mgr. per cc.

(2) The original broth contained $1{\cdot}74 \times 10^{-5}$ mgr. Ra per cc. Inoculated with *living Staph. pyog. aur.* and allowed to incubate at 37° C. for 12 days, the centrifuged deposit (·35 cc.) contained radium at the rate of $6{\cdot}8 \times 10^{-3}$ mgr. per cc., and the supernatant broth corrected for evaporation contained $1{\cdot}069 \times 10^{-6}$ mgr. per cc.

(3) The original broth contained $1{\cdot}204 \times 10^{-5}$ mgr. Ra per cc. Allowed to grow aerial bacteria of any kind at room temperature; the deposit (2·017 cc.) contained radium at the rate of $1{\cdot}84 \times 10^{-4}$ mgr. per cc., while the supernatant broth corrected for evaporation contained $1{\cdot}106 \times 10^{-5}$ per cc.

(4) The original broth contained $1{\cdot}314 \times 10^{-6}$ mgr. Ra per cc. Allowed to grow aerial bacteria of any kind at room temperature: the deposit (1·426 cc.) contained radium at the rate of $2{\cdot}74 \times 10^{-5}$ mgr. per cc., while the supernatant broth corrected for evaporation contained $8{\cdot}04 \times 10^{-7}$ mgr. per cc.

Taking the amount of radium in the broth at the commencement of the experiment as unity in each of the above experiments, the relative amounts per cc. in bacterial deposit and in supernatant broth at the end of the experiments were as follows:—

	Experiment 1.	Experiment 2.	Experiment 3.	Experiment 4.
Bacterial deposit	247	385	15	21
Broth	0·58	0·061	0·9	0·6

The subject is undergoing further investigation.

Correlation of the amounts of Radium in Gallstones with the frequency of the occurrence of Gallstones in Carcinoma.

In the present section a comparison is made between the frequency with which gallstones occur in different classes of patient and the amounts of radium present in such gallstones.

At the Middlesex Hospital, during the years 1900–4 inclusive, autopsies were made on 1448 individuals above the age of 35 years. Of these, 699 were carcinomatous, 749 non-malignant cases.

Amongst the 749 non-malignant cases gallstones were found on 37 occasions, i.e. 4·94 %. Dividing the 699 carcinoma cases into two classes, viz., those having primary carcinoma of the gall-bladder and those having carcinoma at any other site, 693 cases of carcinoma at sites other than the gall-bladder afforded gallstones on 59 occasions, i.e. 8·51 %, and 6 cases of primary carcinoma of the gall-bladder afforded gallstones on 6 occasions, i.e. 100 %. It is possible that the last percentage is somewhat too high, for Colwell,* dealing with a period of 50 years, states, "gallstones have been discovered at the post-mortem examination in 27 of the 31 cases of primary malignant disease of the gall-bladder and bile-passages at the Middlesex Hospital. In the remaining 4 cases of this series it is specifically stated that no gallstones were discovered. No mention, however, is made on the point of pigmentary debris." Colwell's figures give the percentage occurrence of gallstones in primary carcinoma of the gall-bladder as 87·1.

Taking now the radium contents of gallstones corresponding to the same groups of cases it is found that the mean amounts of Ra per gram of gallstone as measured by the ether-extraction method are $12{\cdot}7\times10^{-10}$ mgr. for the non-malignant group, $47{\cdot}9\times10^{-10}$ mgr. for the group of carcinoma cases at primary sites other than the gall-bladder, and $314{\cdot}3\times10^{-10}$ per gram of gallstone found associated with primary carcinoma of the gall-bladder. Taking values obtained by the incineration method the mean amounts of Ra per gram of gallstone are 0 for the non-malignant group, $2{\cdot}1\times10^{-10}$ mgr. for the group of carcinomata at sites other than the gall-bladder, and 468×10^{-10} mgr. per gram of gall-

* Colwell, "Arch. Middlesex Hosp., Fourth Cancer Report," 1905, p. 133. Cf. also Ibid. p. 142–8.

stone found associated with primary carcinoma of the gall-bladder. These values are set side by side below.

Cases.	Frequency of gallstones per cent.	Amount of Ra per gram gallstone ($\times 10^{-10}$ mgr.)	
		Ether-extraction method.	Incineration method.
Non-malignant ...	4·94	12·7	0
Carcinoma primary at sites other than gall-bladder ...	8·51	47·9	2·1
Primary carcinoma of gall-bladder ...	100 or 87·1	314·3	468

A glance at these series shows their general similarity, and in particular indicates that that variety of gallstone which is associated with primary carcinoma of the gall-bladder—though indistinguishable by the naked eye or by ordinary chemical tests from other varieties of gallstones not associated with primary carcinoma of the gall-bladder—is distinguished by possessing a relatively considerable amount of radium. This fact suggests that the radium contained in these gallstones is closely bound up with the occurrence of carcinoma around them, and may be considered along with the fact that the author has already shown (p. 91 and fol.), that radium is to be found in some carcinomatous tumours.

Note.—Since writing this paper two other specimens of gallstone have been examined for radium. No. 843 consisted of 5·55 gm. of impure cholesterin gallstones contained in a much-thickened gall-bladder removed by operation. Macroscopically the gall-bladder strongly suggested carcinoma, but microscopically chronic inflammation alone was found. No radium was found in the gallstones or in the gall-bladder (No. 842), which were separately examined for radium by the emanation method. Incineration was used in both instances. No. 846 consisted of a single pigment gallstone weighing ·31 gm. from the body of a man dead of primary carcinoma of the liver encircling the gall-bladder. The mucous membrane and wall of the gall-bladder were intact, and the latter was sharply definable from the surrounding growth. No radium was found in the gallstone, but, as will be seen by reference to p. 96, small quantities of radium were found in the carcinomatous tissue and in the hepatic tissue itself (Nos. 847 and 848 loc. cit.). The results are in accord with the author's findings as expressed in the body of the paper.

PROTOCOLS OF EXPERIMENTS.

(For Protocol of Estimation of Radium Standard, see p. 97.)

February 20th, 1912.

No. 799.—·8 gm. of impure cholesterin gallstone from a case of primary carcinoma of gall-bladder; cholesterin extracted three times with ether (anæsthetic); residue boiled with 10 cc. stock HCl.

9.50 a.m. Natural leak 70th–65th division on scale 22′ 48″ = 220 div./min.
10.43–11.2 a.m. Boiled flask and ran gas into electroscope.

				Rate div./min.	Estim'd N.L. div./min.	Leak due to substance. div./min.
11. 3 a.m.	Leak taken 70th–65th div. on scale		8′ 20″			
11.14 a.m.	Repeated	,, ,,	8′ 38·4″			
11.25 a.m.	Repeated	,, ,,	8′ 40·2″			
11.35 a.m.	Repeated	,, ,,	8′ 11·2″	·606	*less* ·205	= ·401
11.45 a.m.	Repeated	,, ,,	7′ 47″			
11.55 a.m.	Repeated	,, ,,	7′ 57″			
12. 5 p.m.	Repeated	,, ,,	7′ 44″			
12.16 p.m.	Repeated	,, ,,	7′ 56″			
12.33 p.m.	Repeated	,, ,,	7′ 21·2″			
12.43 p.m.	Repeated	,, ,,	7′ 29·4″	·661	,, ·195	= ·466
12.52 p.m.	Repeated	,, ,,	7′ 19·8″			
1. 0 p.m.	Repeated	,, ,,	7′ 35″			
2.10 p.m.	Repeated	,, ,,	8′ 49″			
2.22 p.m.	Repeated	,, ,,	7′ 45″			
2.32 p.m.	Repeated	,, ,,	7′ 35·4″	·651	,, ·175	= ·476
2.43 p.m.	Repeated	,, ,,	7′ 44″			
2.52 p.m.	Repeated	,, ,,	7′ 39·8″			
	Electroscope exhausted twice.					
3.32 p.m.	Natural leak 70th–65th div. on scale		16′ 53·4″			
3.54 p.m.	Repeated	,, ,,	21′ 27·4″	·292	,, ·161	= ·131
4.16 p.m.	Repeated	,, ,,	24′ 36″			
4.44 p.m.	Repeated	,, ,,	26′ 6″	·197	,, ·152	= ·045
5.11 p.m.	Repeated	,, ,,	27′ 47″			
5.39 p.m.	Repeated	65th–60th ,,	35′ 13″	·161	,, ·144	= ·017

Leak due to substance calculated to Ra standard = $4{\cdot}7494 \times 10^{-8}$ mgr. Ra.

April 26th, 1912.

No. 799A.—3 gm. of similar gallstones from above case incinerated direct in platinum dish previously washed out with stock HCl; residue dissolved in 1 cc. stock HCl.

7.3 p.m. Natural leak 70th–65th division on scale 45′ 38″ = ·110 div./min.
7.51–8.5 p.m. Boiled flask and let gas into reservoir of electroscope.

8. 7 p.m.	Leak taken 70th–65th division on scale		3′ 32″	
8.20 p.m.	Repeated	,, ,,	3′ 13·6″	1·552 div./min.
8.27 p.m.	Repeated	,, ,,	3′ 13″	
8.31 p.m.	Repeated	,, ,,	3′ 11·6″	

Radium estimated on leak obtained 20 minutes after letting gas into electroscope calculated to Ra standard = $7{\cdot}17 \times 10^{-8}$ mgr. Ra per gram of gallstone.

February 19th, 1912.

No. 800.—5 gm. of impure cholesterin gallstone from a case of primary carcinoma of the gall-bladder. Extracted with ether three times. Residue boiled with 10 cc. stock HCl.

9.44 a.m. Natural leak 70th–65th division on scale 23′ 51·6″ = ·210 div./min.
10.20–10.41 a.m. Flask boiled and gas let into reservoir of electroscope.

					Leak div./min.	Estimated N.L. div./min.	Leak of substance div./min
10.42 a.m.	Leak taken	70th–65th div.	on scale	5′ 1·8″			
10.49 a.m.	Repeated	„	„	4′ 50·2″			
10.54 a.m.	Repeated	„	„	4′ 48·4″			
11. 3 a.m.	Repeated	„	„	4′ 55″			
11.10 a.m.	Repeated	„	„	4′ 31″	1·076	*less* ·199	= ·877
11.17 a.m.	Repeated	„	„	4′ 32·2″			
11.24 a.m.	Repeated	„	„	4′ 35·2			
11.31 a.m.	Repeated	„	„	4′ 30·2			
11.40 a.m.	Repeated	„	„	4′ 12·″			
11.45 a.m.	Repeated	„	„	4′ 1″			
11.51 a.m.	Repeated	„	„	4′ 8·4″			
11.57 a.m.	Repeated	„	„	4′ 11·4″			
12. 4 p.m.	Repeated	„	„	4′ 0″			
12.10 p.m.	Repeated	„	„	3′ 55·2″	1·265	„ ·189	= 1·076
12.15 p.m.	Repeated	„	„	3′ 49·4″			
12.22 p.m.	Repeated	„	„	3′ 49·4″			
12.27 p.m.	Repeated	„	„	3′ 55″			
12.37 p.m.	Repeated	„	„	3′ 47·2″			
12.46 p.m.	Repeated	„	„	3′ 46·8″			
12.55 p.m.	Repeated	„	„	3′ 51·6″			
1. 3 p.m.	Repeated	„	„	3′ 56″			
1. 9 p.m.	Repeated	„	„	3′ 50·4″			
1.18 p.m.	Repeated	„	„	3′ 47·6″	1·314	„ ·179	= 1·135
1.24 p.m.	Repeated	„	„	3′ 47·6″			
1.32 p.m.	Repeated	„	„	3′ 53·2″			
1.38 p.m.	Repeated	„	„	3′ 45″			
		Electroscope exhausted twice.					
2.21 p.m.	Natural leak	70th–65th div.	on scale	10′ 16·4″			
2.35 p.m.	Repeated	„	„	12′ 54·6″			
2.50 p.m.	Repeated	„	„	16′ 40·4″	·357	„ ·170	= ·187
3. 8 p.m.	Repeated	„	„	20′ 36″			
3.33 p.m.	Repeated	„	„	24′ 53″	·190	„ ·160	= ·030
4. 0 p.m.	Repeated	„	„	27′ 52″			
4.29 p.m.	Repeated	65th–51st	„	97′ 26″	·144	„ ·144	= 0

Leak due to substance calculated to Ra standard = 1·1 × 10^{-7} mgr. Ra.

April 26th, 1912.

No. 800A.—3 gm. of similar gallstones from above case incinerated direct in platinum dish; residue dissolved in 1 cc. stock HCl.

2.55 p.m. Natural leak 70th–65th division on scale 62′ 0″ = ·0807 div./min.
3.57–4.13 p.m. Boiled flask and let gas into reservoir of electroscope.
4.14 p.m. Leak taken 70th–65th division on scale 6′ 18″
4.24 p.m. Repeated 70th–60th „ „ 12′ 27″
4.37 p.m. Repeated 70th–65th „ „ 5′ 31″ = ·9063 div./min.
4.45 p.m. Repeated „ „ „ 5′ 18″

Radium estimated on leak obtained 20 minutes after letting gas into electroscope, calculated to Ra standard = 4·10 × 10^{-5} mgr. Ra per gram of gallstone.

March 11th, 1912.

No. 810.—An impure cholesterin gallstone weighing 1·2259 gm. removed by operation with the gall-bladder, which proved microscopically to be the seat of carcinoma. Ether-extracted three times. Residue boiled with stock HCl.

10.18 a.m. Natural leak 70th–65th division on scale 48′ 52″ = ·102 div./min.
10.48–11.7 a.m. Boiled flask and ran gas into reservoir.

			Leak div./min.	Estim'd N.L. div./min.	Leak due to substance div./min.
11. 9 a.m.	Leak taken 70th–65th div. on scale	23′ 56″	·213	*less* ·102	= ·111
11.36 a.m.	Repeated ,, ,,	22′ 55·6″			
12. 2 p.m.	Repeated ,, ,,	19′ 33″	·243	,, ·102	= ·141
12.24 p.m.	Repeated ,, ,,	21′ 44″			
12.55 p.m.	Repeated ,, ,,	18′ 50″	·265	,, ·102	= ·163
1.14 p.m.	Repeated ,, ,,	17′ 59″			
1.35 p.m.	Repeated ,, ,,	19′ 45″			
	Electroscope exhausted twice.				
2.58 p.m.	Natural leak 70th–65th div. on scale	36′ 2″	·139	,, ·102	= ·037
3.37 p.m.	Repeated ,, ,,	40′ 56·2″	·122	,, ·102	= ·020
4.21 p.m.	Repeated ,, ,,	49′ 28″	·101	,, ·101	= 0

Leak of substance calculated to Ra standard indicates $1·58 \times 10^{-8}$ mgr. Ra.

February 22nd, 1912.

No. 801.—1·02 gm. of impure cholesterin gallstones from a case of carcinoma of breast. Ether-extracted three times. Residue boiled with 10 cc. stock HCl.

9.47 a.m. Natural leak 70th–65th division on scale 26′ 39·6″ = ·188 div./min.
10.25–10.45 a.m. Boiled flask and ran gas into reservoir of electroscope.

			Leak div./min.	Estim'd N.L. div./min.	Leak due to substance div./min.
10.45 a.m.	Leak taken 70th–65th div. on scale	15′ 21″			
11. 1 a.m.	Repeated ,, ,,	14′ 57″	·331	*less* ·182	= ·149
11.17 a.m.	Repeated ,, ,,	15′ 7·4″			
11.35 a.m.	Repeated ,, ,,	15′ 5″			
11.52 a.m.	Repeated ,, ,,	14′ 43·3″			
12.11 p.m.	Repeated ,, ,,	14′ 22·4″	·356	,, ·178	= ·178
12.29 p.m.	Repeated ,, ,,	13′ 42·6″			
12.44 p.m.	Repeated ,, ,,	13′ 32·4″			
1. 0 p.m.	Repeated ,, ,,	13′ 42″			
1.15 p.m.	Repeated ,, ,,	13′ 5·6″	·365	,, ·174	= ·191
1.30 p.m.	Repeated ,, ,,	13′ 55·4″			
1.47 p.m.	Repeated ,, ,,	14′ 10·2″			
	Electroscope exhausted twice.				
2.32 p.m.	Natural leak 70th–65th div. on scale	22′ 46·8″	·210	,, ·168	= ·042
3. 1 p.m.	Repeated ,, ,,	24′ 46·2″			
3.29 p.m.	Repeated ,, ,,	27′ 17″	·178	,, ·164	= ·014
3.58 p.m.	Repeated ,, ,,	28′ 40″			
4.27 p.m.	Repeated 65th–59th ,,	38′ 5″	·158	,, ·158	= 0
5.22 p.m.	Repeated 69th–64th ,,	31′ 17″			

Leak due to substance calculated to Ra standard = $1·851 \times 10^{-8}$ mgr. Ra.

April 26th, 1912.

No. 801A.—1·5 gm. of similar gallstones from same case as above, incinerated in platinum dish. Residue dissolved in 1 cc. stock HCl.

10.58 a.m. Natural leak 70th–65th division on scale, 49′ 51″ = ·100 div./min.
11.55 a.m.–12.7 p.m. Boiled flask and ran gas into reservoir of electroscope.
12. 8 p.m. Leak taken 70th–65th division on scale, 52′ 46″ = ·095 div./min.
No evidence of radium.

February 2nd, 1912.

No. 802.—1·33 gm. of impure cholesterin gallstone from a case of carcinoma of the urinary bladder. Extracted with ether three times. Residue boiled with 10 cc. stock HCl.

9.50 a.m. Natural leak 70th–65th division on scale, 26′ 35″ = ·188 div./min.
10.28–10.49 a.m. Flask boiled; gas run into reservoir of electroscope.
10.49 a.m. Leak taken 70th–65th division on scale 25′ 8″
11.15 a.m. Repeated „ „ 30′ 38″ } ·184 div./min.
11.49 a.m. Repeated „ „ 26′ 25″
No evidence of radium.

March 7th, 1912.

Above repeated.

11.14 a.m. Natural leak 70th–64th division on scale, 62′ 37″ = ·096 div./min.
12.16 p.m.–12.32 p.m. Boiled flask and ran gas into reservoir of electroscope.

			Leak div./min.	Estim'd N.I.. div./min.	Leak due to substance div./min.
12.37 p.m.	Leak taken 70th–65th div. on scale	50′ 58″	= ·098	*less* ·101	= −·003
1.31 p.m.	Repeated 70th–64th „	53′ 0·2″	= ·113	„ ·103	= +·010
2.32 p.m.	Repeated 70th–65th „	42′ 48″	= ·117	„ ·106	= +·011
	Electroscope exhausted twice.				
3.49 p.m.	Natural leak 70th–65th div. on scale	39′ 50·2″	= ·126	„ ·109	= +·017
5.18 p.m.	Repeated „ „	44′ 8·4″	= ·113	„ ·114	= −·001
6. 8 p.m.	Repeated 75th–60th „	33′ 13″	= ·150	„ ·118	= +·032

Mean of all natural leak observations = ·109 div./min.
„ experimental „ = ·109 „
No evidence of radium.

March 25th, 1912.

No. 802A.—3 gm. of similar gallstones from same case as above. Incinerated in platinum dish. Residue dissolved in 1 cc. stock HCl.

10.39 a.m. Natural leak 70th–65th division on scale, 42′ 57·2″ = ·116 div./min.
11.32–11.50 a.m. Boiled flask and ran gas into reservoir of electroscope.
11.50 a.m. Leak taken 70th–65th division on scale, 47′ 30″ = ·105 div./min.
No evidence of radium.

February 22nd, 1912.

No. 803.—2·185 gm. of impure cholesterin gallstone from a case of carcinoma of vulva. Ether-extracted three times. Residue boiled with 10 cc. stock HCl.

9.50 a.m. Natural leak 70th–65th division on scale 26′ 35″ = ·188 div./min.
8. 0 p.m. Repeated „ „ 30′ 29·8″ = ·145 div./min.
Estimated natural leak at 12.45 = ·176 div./min.
12.22–12.44 p.m. Boiled flask and ran gas into electroscope.
12.45 p.m. Leak taken 70th–65th division on scale 29′ 49″ = ·168 div./min.
1.16 p.m. Repeated „ „ 33′ 59″ = ·147 div./min.
1.52 p.m. Repeated 70th–63rd „ 47′ 55″ = ·146 div./min.
No evidence of radium.

April 25th, 1912.

No. 803A.—3 gm. of similar gallstones from above case. Incinerated in platinum dish. Residue dissolved in 1 cc. stock HCl.

10.39 a.m. Natural leak 70th–65th division on scale 42′ 57·2″ = ·116 div./min.
5.46 p.m. Repeated „ „ 54′ 22″ = ·092 div./min.
Estimated natural leak at 12.55 p.m. = ·110 div./min.
12.40–12.52 p.m. Boiled flask and ran gas into reservoir of electroscope.
12.55 p.m. Leak taken 70th–65th division on scale 51′ 43″ = ·097 div./min.
No evidence of radium.

February 22nd, 1912.

No. 804.—2·2425 gm. of impure cholesterin gallstone from a case of carcinoma of the rectum. Ether-extracted three times. Residue boiled with 10 cc. stock HCl.

9.50 a.m. Natural leak 70th–65th division on scale 31′ 24″ = ·159 div./min.
10.32–10.53 a.m. Boiled flask and ran gas into reservoir of electroscope.

			Leak div./min.	Estim'd. N.L. div./min.	Leak due to substance. div./min.
10.54 a.m.	Leak taken 70th–65th div. on scale	22′ 30″	·216	*less* ·158	= ·058
11.17 a.m.	Repeated „ „	23′ 42″			
11.44 a.m.	Repeated „ „	22′ 52·8″			
12. 8 p.m.	Repeated „ „	21′ 31″	·230	„ ·157	= ·073
12.32 p.m.	Repeated „ „	21′ 0″			
12.55 p.m.	Repeated „ „	20′ 54″			
1.17 p.m.	Repeated „ „	21′ 30″	·234	„ ·156	= ·078
1.41 p.m.	Repeated „ „	21′ 44·4″			
	Electroscope exhausted once.				
2.30 p.m.	Natural leak 70th–65th div. on scale	28′ 41″			
3. 0 p.m.	Repeated „ „	30′ 19″	·169	„ ·154	= ·015
3.35 p.m.	Repeated „ „	31′ 13″			
4.10 p.m.	Repeated „ „	32′ 43″	·156	„ ·153	= ·003
5.11 p.m.	Repeated „ „	32′ 55″	·152	„ ·152	= 0

Leak of substance calculated to Ra standard indicates $8·044 \times 10^{-9}$ mgr. Ra.

April 25th, 1912.

No. 804A.—3 gm. of similar gallstones from same case as above. Incinerated in platinum dish. Residue dissolved in 1 cc. stock HCl.

10.39 a.m. Natural leak 70th–65th division on scale 42′ 57·2″ = ·116 div./min.
5.46 p.m. Repeated „ „ 54′ 22″ = ·092 „
Estimated natural leak at 4.20 p.m., ·097 div./min.
4.4–4.18 p.m. Boiled flask and let gas into reservoir of electroscope.
4.21 p.m. Leak taken 70th–65th division on scale 44′ 56″ = ·112 div./min.
Leak of substance calculated to Ra standard indicates $1{\cdot}87 \times 10^{-9}$ mgr. Ra.

March 12th, 1912.

No. 811.—1·403 gm. of impure cholesterin gallstone from a case of carcinoma of the stomach. Ether-extracted three times. Residue boiled with 10 cc. stock HCl.

10.8 a.m. Natural leak 70th–65th division on scale 51′ 29″ = ·097 div./min.
11.3–11.19 a.m. Boiled flask and ran gas into reservoir of electroscope.

			Leak. div./min.	Estim'd N.L. div./min.	Leak due to substance. div./min.
11.22 a.m.	Leak taken 70th–65th div. on scale	38′ 54″			
12. 3 p.m.	Repeated „ „	39′ 48″	·127	*less* ·097	= ·030
12.46 p.m.	Repeated „ „	37′ 21·4″			
1.27 p.m.	Repeated „ „	41′ 6″	·128	„ ·097	= ·031
2.11 p.m.	Repeated „ „	38′ 37″	·129	„ ·097	= ·032
	Electroscope exhausted twice.				
3.37 p.m.	Natural leak 70th–64th div. on scale	55′ 42″	·108	„ ·097	= ·011
4.36 p.m.	Repeated 70th–65th „	51′ 22″	·097	„ ·097	= 0
5.30 p.m.	Repeated „ „	47′ 5″	·106	„ ·097	= ·009

Leak of substance calculated to Ra standard = $3{\cdot}101 \times 10^{-9}$ mgr. Ra.

February 22nd, 1912.

No. 805.—1·88 gm. of impure cholesterin gallstone from a non-malignant case. Ether-extracted three times. Residue boiled with 10 cc. stock HCl.

9.50 a.m. Natural leak 70th–65th division on scale 26′ 35″ = ·188 div./min.
8. 0 p.m. Repeated „ „ 34′ 29·8″ = ·145 „
Estimated natural leak at 3 p.m. = ·164 div./min.
2.51–3.13 p.m. Boiled flask and let gas into reservoir of electroscope.

			Leak. div./min.	Estim'd N.L. div./min.	Leak due to substance. div./min.
3.14 p.m.	Leak taken 70th–65th div. on scale	31′ 6″			
3.47 p.m.	Repeated „ „	30′ 30″	·163	*less* ·163	= 0
4.12 p.m.	Repeated „ „	29′ 22·4″			
4.51 p.m.	Repeated „ „	29′ 51·4″	·168	„ ·158	= ·010
5.22 p.m.	Repeated „ „	29′ 45″			
5.56 p.m.	Repeated „ „	31′ 43″	·163	„ ·153	= ·010
	Electroscope exhausted once.				
6.30 p.m.	Natural leak 70th–65th div. on scale	30′ 27″	·164	„ ·149	= ·015
7.22 p.m.	Repeated „ „	34′ 54″	·143	„ ·147	= –·004
8. 0 p.m.	Repeated „ „	34′ 29·8″	·145	„ ·143	= ·002
	No sufficient evidence of radium.				

April 25th, 1912.

No. 805A.—3 gm. of similar gallstones from same case as above. Incinerated in platinum dish. Residue dissolved in 1 cc. stock HCl.

10.39 a.m. Natural leak 70th–65th division on scale 42′ 57·2″ = ·116 div./min.
5.46 p.m. Repeated „ „ 54′ 22″ = ·092 „
Estimated natural leak at 2 p.m. = ·107 div./min.
1.50–2.0 p.m. Boiled flask and let gas into reservoir of electroscope.
2. 0 p.m. Leak taken 70th–65th division on scale 49′ 44″ = ·101 div./min.
No evidence of radium.

March 6th, 1912.

No. 809.—2·39 gm. of impure cholesterin gallstone derived from various non-malignant cases. Ether-extracted three times. Residue boiled with 10 cc. stock HCl.

10.59 a.m. Natural leak 70th–65th division on scale 48′ 22″ = ·103 div./min.
11.50 a.m.–12.6 p.m. Boiled flask and ran gas into reservoir of electroscope.

				Leak div./min.		N.L. div./min.	Estim'd substance, Leak due to div./min.
12. 6 p.m.	Leak taken	70th–65th div. on scale	31′ 45″	·157	*less*	·102	= ·055
12.41 p.m.	Repeated	„ „	33′ 36″	·170	„	·101	= ·069
1.18 p.m.	Repeated	„ „	26′ 0″				
1.55 p.m.	Repeated	70th–63rd „	43′ 28″	·177	„	·100	= ·077
2.43 p.m.	Repeated	70th–65th „	25′ 51·4″				
		Electroscope exhausted twice.					
3.43 p.m.	Natural leak	70th–65th div. on scale	40′ 15″	·124	„	·099	= ·025
4.26 p.m.	Repeated	„ „	44′ 38″	·112	„	·098	= ·014
5.15 p.m.	Repeated	„ „	51′ 58″	·096	„	·096	= 0

Leak of substance calculated to Ra standard = $7{\cdot}46 \times 10^{-9}$ mgr. Ra.

April 25th, 1912.

No. 809A.—3 gm. of similar gallstones to No. 809. Incinerated in platinum dish. Residue dissolved in 1 cc. stock HCl.

10.39 a.m. Natural leak 70th–65th division on scale 42′ 57·2″ = ·116 div./min.
5.46 p.m. Repeated „ „ 54′ 22″ = ·092 „
Estimated natural leak at 3.30 p.m. = ·100 div./min.
2.51–3.7 p.m. Boiled flask and ran gas into reservoir of electroscope.
3.8 p.m. Leak taken 70th–65th division on scale 51′ 53″ = ·096 div./min.
No evidence of radium.

March 15th, 1912.

No. 812.—3·37 gm. of impure cholesterin gallstone from a non-malignant case. Ether-extracted three times. Residue boiled with 10 cc. stock HCl.

10.34 a.m. Natural leak 70th–65th division on scale, 45′ 51″ = ·109 div./min.
11.23–11.35 a.m. Boiled flask and ran gas into reservoir of electroscope.

			Leak div. min.	Estim'd N.L. div. min.	Leak due to substance div. min.
11.53 a.m.	Leak taken 70th–65th div. on scale	40′ 24·6″	·126	*less* ·106	= ·020
12.38 p.m.	Repeated „ „	36′ 47″	·133	„ ·106	= ·033
1.24 p.m.	Repeated „ „	38′ 25″	·130	„ ·098	= ·035
2.55 p.m.	Repeated „ „	36′ 40″	·136		
	Electroscope exhausted twice.				
4.13 p.m.	Natural leak 70th–65th div. on scale	42′ 52·4″	·117	„ ·093	= ·024
5. 3 p.m.	Repeated „ „	53′ 54″	·093	„ ·090	= ·003
6. 0 p.m.	Repeated „ „	56′ 25″	·088	„ ·088	= 0

Leak of substance calculated to Ra standard indicates $3{\cdot}39 \times 10^{-9}$ mgr. Ra.

March 13th, 1912.

No. 813.—5·3 gm. of impure cholesterin gallstone from a non-malignant case. Ether-extracted three times. Residue boiled with 10 cc. stock HCl.

10. 4 a.m. Natural leak 70th–65th division on scale, 52′ 31″ = ·095 div./min.
11. 3–11.20 a.m. Boiled flask and ran gas into reservoir of electroscope.

			Leak div./min.	Estim'd N.L. div./min.	Leak due to substance div./min.
11.22 a.m.	Leak taken 70th–65th div. on scale	43′ 29·6″	·115	*less* ·095	= ·020
12.18 p.m.	Repeated „ „	47′ 33″	·105	„ ·095	= ·010
1.25 p.m.	Repeated „ „	50′ 1·4″	·100	„ ·096	= ·004
	Electroscope exhausted twice.				
2.53 p.m.	Natural leak 70th–65th div. on scale	42′ 20″	·118	„ ·097	= ·021
3.43 p.m.	Repeated „ „	49′ 52″	·100	„ ·097	= ·003
4.38 p.m.	Repeated „ „	51′ 14″	·098	„ ·098	= 0

No evidence of radium.

March 21st, 1912.

No. 821. –1·067 gm. of pigment gallstones from a non-malignant case. Ether-extracted three times. Residue boiled with 10 cc. stock HCl.

9.48 a.m. Natural leak 70th–65th division on scale, 47′ 7″ = ·106 div./min.
10.37–10.57 a.m. Boiled flask and ran gas into reservoir of electroscope.

			Leak div. min.	Estim'd N.L. div./min.	Leak due to substance div./min.
11. 0 a.m.	Leak taken 70th–65th div. on scale	37′ 26″	·134	*less* ·105	= ·029
11.45 a.m.	Repeated „ „	36′ 50″	·136	„ ·104	= ·032
12.23 p.m.	Repeated „ „	35′ 48″	·134	„ ·102	= ·032
1.11 p.m.	Repeated „ „	39′ 5″			
	Electroscope exhausted twice.				
2.24 p.m.	Natural leak 70th–65th div. on scale	44′ 24″	·113	„ ·101	= ·012
3.15 p.m.	Repeated „ „	49′ 32″	·101	„ ·100	= ·001
4. 8 p.m.	Repeated „ „	50′ 13″	·099	„ ·099	= 0

Leak calculated to Ra standard indicates $3{\cdot}1 \times 10^{-9}$ mgr. Ra.

March 22nd, 1912.

No. 822.—4·628 gm. of impure cholesterin gallstone from a non-malignant case. Extracted with ether three times. Residue boiled with 10 cc. stock HCl.

10.55 a.m. Natural leak 70th–65th division on scale 49′ 18″ = ·100 div./min.
11.43–12.2 p.m. Boiled flask and ran gas into reservoir of electroscope.

		Leak div./min.	Estimated N.L. div./min.	Leak due to substance div./min.
12. 3 p.m.	Leak taken 70th–65th div. on scale 42′ 59″	·116	*less* ·098	= ·022
12.50 p.m.	Repeated „ „ 40′ 0″	·125		
1.44 p.m.	Repeated „ „ 37′ 46·5″	·132	„ ·097	= ·031
2.35 p.m.	Repeated „ „ 44′ 53″	·111	„ ·096	= ·025

Electroscope exhausted twice.

4. 4 p.m.	Natural leak 70th–65th div. on scale 44′ 32″	·112	„ ·093	= ·019
4.58 p.m.	Repeated „ „ 54′ 34″	·092	„ ·092	= 0

Leak of substance calculated to Ra standard indicates $2{\cdot}71 \times 10^{-9}$ mgr. Ra.

May 13th, 1912.

No. 843.—5·55 gm. of impure cholesterin gallstone from an operation case, with greatly thickened (inflammatory) gall-bladder. No trace of malignant disease microscopically. Incinerated in platinum dish. Residue dissolved in 1 cc. stock HCl.

2 p.m. Natural leak 70th–63rd division on scale 80′ 49″ = ·087 div./min.
3.26–3.42 p.m. Boiled flask and let gas into reservoir of electroscope.
3.42 p.m. Leak taken 70th–65th division on scale 62′ 31″ = ·087 div./min.
4.46 p.m. Repeated „ „ 57′ 54″ = ·086 „
No evidence of radium.

June 6th, 1912.

No. 846.—·31 gm. of pigment gallstone from a case of primary malignant disease of the liver encircling the gall-bladder, but not macroscopically involving it. Incinerated in platinum dish. Residue dissolved in 1 cc. stock HCl.

12.19 p.m. Natural leak 70th–63rd division on scale 64′ 40″
1.28 p.m. Repeated 70th–61st „ „ 91′ 53″ } ·102 div./min.
3.0–3.21 p.m. Boiled flask and let gas into reservoir of electroscope.

3.24 p.m.	Leak taken 70th–65th div. on scale 47′ 36″	·105 div./min.	= ·101 div./min.
4.14 p.m.	Repeated 70th–64th „ 61′ 28″	·097 „	
5.17 p.m.	Repeated 69th–67th „ 20′ 4″	·100 „	

Electroscope exhausted once.

6. 5 p.m. Natural leak 67th–65th div. on scale 20′ 16″ = ·100 div./min.
No evidence of radium.

April 27th, 1912.

No. 827.—6·333 gm. of cholesterin removed by ether extraction from the various gallstones extracted with ether (mixed). Incinerated in platinum dish. Residue dissolved in 1 cc. stock HCl.

11.32 a.m. Natural leak 70th–61st division on scale 123′ = ·0732 div./min.
3.0–3.18 p.m. Boiled flask and ran gas into reservoir of electroscope.
3.22 p.m. Leak taken 70th–67th division on scale 40′ 7″ = ·0714 div./min.

April 30th, 1912.

The experiment was repeated after 3 days' possible accumulation of radium emanation, by which time it should have attained 41 per cent. of equilibrium value.

3.4 p.m. Natural leak 70th–63rd division on scale 65′ 30″ = ·107 div./min.
4.30–4.45 p.m. Flask boiled and gas let into reservoir of electroscope.
4.48 p.m. Leak taken 70th–63rd division on scale 69′ 40″ = ·100 div./min.

No evidence of radium.

March 5th, 1912.

10 cc. stock nutrient broth + 10 cc. stock HCl.

3.43 p.m. Natural leak 70th–65th division on scale 34′ 39″ = ·144 div./min.
4.27–4.47 p.m. Boiled flask and ran gas into reservoir of electroscope.

			Leak div./min.	N.L. div./min.	Leak due to substance. div./min.
4.48 p.m.	Leak taken 70th–65th div. on scale	8′ 34·6″			
4.59 p.m.	Repeated „ „	7′ 13·8″			
5.10 p.m.	Repeated „ „	7′ 46″	·652	*less* ·144	= ·508
5.22 p.m.	Repeated „ „	7′ 49·8″			
5.34 p.m.	Repeated „ „	6′ 44″			
5.44 p.m.	Repeated „ „	6′ 53·4″			
6.18 p.m.	Repeated „ „	6′ 47·6″	·737	„ ·144	= ·593
6.31 p.m.	Repeated „ „	6′ 41″			
6.43 p.m.	Repeated „ „	6′ 36″			
7. 3 p.m.	Repeated „ „	6′ 40·4″	·754	„ ·144	= ·610
7.55 p.m.	Repeated „ „	6′ 38·4″			

Electroscope exhausted twice.

9. 3	Natural leak 70th–66th division on scale	22′ 37″	·177	„ ·144	= ·033
9.29	Repeated 70th–65th „	31′ 29″	·159	„ ·144	= ·015

Leak of substance calculated to Ra standard indicates $5{\cdot}91 \times 10^{-9}$ mgr. Ra per 1 cc. broth.

March 4th, 1912.

3 cc. of stock nutrient broth to which $RaCl_2$ has been added to form "radium broth." 10 cc. stock HCl. Condensed protocol.

10.41 a.m. Natural Leak 70th–65th div. on scale 20′ 48″ } ·243 div./min.
11. 8 a.m. Repeated „ „ 20′ 23·2″ } Bar. press. 748 mm. Hg.
11.43–11.58 a.m. Boiled flask and let gas into reservoir of electroscope.
11.58 a.m. Leak taken 70th–40th div. on scale 43″ at bar. press. 155 mm. Hg.
12. 0 noon Repeated „ „ 37″ „ „
12. 1 p.m. Repeated „ „ 36″ „ „
Readings repeated steadily; bar. press. rising owing to leak into reservoir.

12.59 p.m.	Leak 70th–40th division on scale	20·8″	
1. 2 p.m.	Repeated „ „	20·8″	Bar. 211 mm. Hg.
1. 3 p.m.	Repeated „ „	20·6″	
1.55 p.m.	Repeated „ „	16″	
1.57 p.m.	Repeated „ „	15·4″	Bar. 258 mm. Hg.
1.59 p.m.	Repeated „ „	15·6″	
2.58 p.m.	Repeated „ „	13·2″	
2.59 p.m.	Repeated „ „	13·2″	Bar. 430 mm. Hg.
3. 0 p.m.	Repeated „ „	13·4″	

Leak of substance calculated to Ra standard and 760 mm. Hg indicates $1{\cdot}74 \times 10^{-5}$ mgr. Ra per cc. "radium broth."

March 23rd, 1912.

No. 814.—5 cc. of centrifuged "radium broth" in which *Staphylococcus pyogenes aureus* had grown 12 days at 37° C. 10 cc. HCl. Condensed protocol.

10.30 a.m. Natural leak 70th–65th division on scale 48′ 2″ = ·104 div./min.
3.50–4.2 p.m. Boiled flask and let gas into reservoir of electroscope.
4. 5 p.m. Leak taken 80th–60th division on scale 4′ 2″ Bar. press. 56 mm. Hg.
4.11 p.m. Repeated 70th–50th „ 4′ 23·4″
4.27 p.m. Repeated „ „ 4′ 38·4″ Bar. press. 62 mm. Hg.

Leak of substance calculated to Ra standard and 760 mm. Hg indicates roughly $1{\cdot}314 \times 10^{-6}$ mgr. Ra in 1 cc.

March 23rd, 1912.

No. 815.—·35 gm. of centrifuged deposit of *Staphylococcus pyogenes aureus* which had grown 12 days in "radium broth." 10 cc. HCl. Condensed protocol.

10.30 a.m. Natural leak as above = ·104 div./min.
5.8–5.22 p.m. Boiled flask and ran gas into reservoir of electroscope.
5.23–5.28 p.m. Leak taken 80th–30th division on scale. Bar. press. 53 mm. Hg. 3·4″, 3·4″, 3·2″, 3·2″
5.38–5.41 p.m. Repeated 100th–0 „ „ Bar. press. 55 mm. Hg. 4·4″, 4·0″, 4·0″, 4·0″

Leak of substance calculated to Ra standard and 760 mm. Hg indicates roughly $6{\cdot}8 \times 10^{-3}$ mgr. Ra per 1 cc. of deposit of bacteria grown in "radium broth."

Note.

5 cc. of the supernatant "radium broth" that had held dead *Staph. pyog. aur.* for 12 days at 37° C. gave a leak of 50 divisions in about 1′ 20″ at barometer pressure 59 mm. Hg, indicating roughly $1{\cdot}2\times10^{-6}$ mgr. Ra per cc.

The centrifuged deposit from above (·15 cc.) gave a leak of 50 divisions in about 4″ at barometric pressure 110 mm., indicating roughly $4{\cdot}3\times10^{-3}$ mgr. Ra per cc.

The two samples of "radium broth" (1 cc. in each case) in which aerial bacteria grew gave respectively 10 divisions in 1′ 23″ (roughly $8{\cdot}7\times10^{-7}$ mgr. Ra) and 40 divisions in 25″ (roughly $1{\cdot}2\times10^{-5}$ mgr. Ra).

The two deposits of bacteria from above gave respectively (1·426 cc.) 100 divisions in 3′ 8″, with barometric pressure 69 mm. Hg (i.e. roughly $2{\cdot}7\times10^{-5}$ mgr. Ra per cc.), and (2·017 cc.) 100 divisions in 22½ seconds, with barometric pressure 67 mm., indicating roughly $1{\cdot}8\times10^{-4}$ mgr. Ra per cc.

Further details are useless, for the values cannot be accurately measured with quantities of radium of these magnitudes by means of the emanation electroscope. They are only given to indicate that the changes noted in the text are marked.

A CONTRIBUTION TO THE STUDY OF THE EFFECTS OF RADIATIONS ON SILKWORMS.

By SOMERVILLE HASTINGS, H. BECKTON, and B. H. WEDD.

The work described in the following pages is a continuation of that recorded in the Ninth Cancer Report (Archives of the Middlesex Hospital, vol. xix.), published in June 1910, and consists of a continuous series of experiments and observations, extending from May 1909 to September 1911, on silkworms for nearly three complete cycles of their life history. X-rays have been applied in all four stages of development in the first or second or in both generations, one of the objects of the research being to find out whether the effects obtained in the first generation could be intensified in the second by a repetition of the same treatment. Slight improvements in technique were introduced in 1910, notably in the removal of the silk before applying X-rays to cocoons, and in the counting of every egg laid—in some instances over a thousand eggs—instead of two hundred only. (Fewer than two hundred were never counted.)

In the following pages are considered the results of apply-

ing *moderate* amounts of radiations to silkworms in various stages as regards

I.—Fertility of eggs,

II.—Date of hatching out of eggs,

III.—Weights of cocoons; *

while the effects of applying *large* doses of radiations to larvæ have also been noted as to

IV.—Weights and vitality of the larvæ themselves at subsequent periods.

I.- EFFECT OF MODERATE DOSES OF RADIATION ON THE FERTILITY OF EGGS.

For the purpose of this research the fertility of eggs produced by silkworms is measured by the percentage from which living larvæ emerge. At the end of the hatching season (early summer) it is easy to recognise an egg from which a larva has hatched out by its paler colour and by the small circular opening in the shell through which the living caterpillar has emerged.

It will be convenient to consider the question of fertility in the cases of—

(A) Eggs themselves irradiated soon after being laid.

(B) Untreated eggs of insects which had been irradiated as larvæ.

(C) Untreated eggs of insects which had been irradiated as pupæ.

(D) Untreated eggs of insects which had been irradiated as moths.

(E) Untreated eggs of insects which had been irradiated in more than one stage of development.

(A)—Exposure of Eggs to Moderate Doses of X-rays.

(1)—(A) On August 10th, 1909, 10 male and 5 female moths emerged from the control chrysalids. These were at once placed in the same box (cf. p. 151). As soon as the females ceased laying, the eggs were collected and X-rays were applied to them for 15 min. each day for three consecutive days—total exposure 45 min. In this and subsequent experiments the same X-ray tube was used; the objects

* The term "cocoon" throughout this paper connotes the silk case together with the contained pupa; and the weight noted was taken in each instance soon after completion of spinning.

X-rayed were placed 5 in. from the anode, and the coil was run with a current of 4·5 amperes, a spark-gap of 5·5 in., and a regulator-gap to the Crookes' tube of 4 in.

(B) On August 11th, 1909, 8 males and 2 females emerged, and some of the resulting eggs were treated in precisely the same way except that four consecutive exposures were given —total 60 min.

In July 1910, when hatching was complete, it was found that 95 per cent. of Series (A) and 84 per cent. of Series (B), average 89·5 per cent., had hatched out as compared with 90·5 per cent. of the controls.

From this it would appear that X-rays are without effect upon newly-laid eggs ; but, as will be seen later, this conclusion is not warranted.

In the spring of 1910, thirty larvæ were selected at random from Series (A) and fed on mulberry leaves. Twenty-nine of them entered the chrysalis stage and five pairs of the moths which emerged were mated separately. 55 per cent. of the resulting eggs were fertile, compared with 91 per cent. in the general controls fed in exactly the same manner. In this experiment, therefore, the effect of small doses of X-rays on silkworms' eggs is only seen in the second generation. Similar evidence of an effect of X-rays upon the second generation is given in the case of other experiments.

(2) In September 1910 a further series of experiments was carried out. Four series of freshly laid eggs were taken and each divided into three lots. One of these served as a control, and to each of the others X-rays were applied for 15 min. and 60 min. respectively on each of three consecutive days. The X-rays were applied to the eggs as they were laid on sheets of paper at a distance of 5 in. from the anode exactly as above described. The results are best shown in the form of a table.

Interval between Mating and first Application of Rays.	Percentage hatched as compared with controls of same series.	
	15 min. exposure for 3 days.	60 min. exposure for 3 days.
5 days	99 per cent.	0 per cent.
7 ,,	71 ,,	0 ,,
11 ,,	11 ,,	0 ,,
13 ,,	86 ,,	0 ,,

As far as this one experiment goes it would appear that X-rays are very destructive to silkworms' eggs if given in large doses, and that in smaller quantity their effects are most marked about the 11th day after their fertilisation. (Active changes resulting in the formation of a well-formed embryo take place during the first two or three weeks after fertilisation, this period of activity being followed by one of quiescence extending over eight or nine months, at the end of which time the larva hatches out.)

(3) In all the above experiments the application of X-rays was made to eggs exactly as laid on pieces of white paper. Now, the silkworm's egg is not completely spherical, but is flattened from above down. Possibly, therefore, the cell contained within the shell may be somewhat flattened from above down, and the first nuclear division may take place regularly either in the transverse or the vertical plane. In either case, if this be so, X-rays applied to the eggs from the side will have a different relation to the chromatic spindle from that of rays falling vertically on them. Three experiments were made with X-rays applied laterally. The papers on which the eggs were laid were cut into crescentic narrow strips so that not more than two or three eggs were lying abreast on each. The strips were mounted so that the plane of the paper was parallel to the rays from the Crookes' tube during exposure, and the crescentic arrangement admitted of an approximately equal exposure of all the cells concerned in the experiment.

When the rays were applied for 60 min. on 3 consecutive days, the first being given 3 days after mating, all the eggs, whether receiving the rays on the edge or on the flat, were rendered sterile; but in a similar experiment (C) made 5 days after mating, although all the eggs receiving X-rays on the flat were unfertile, 31 per cent. of the otherwise fertile eggs which received the rays from the side hatched out. A close examination of these eggs revealed the fact that practically all those eggs nearest to the X-ray tube, and therefore unsheltered from the rays, were rendered sterile. With doses of 15 min. on 3 consecutive days (D), the first being given 11 days after mating, 11 per cent. of the viable eggs hatched out when the rays were received

on the flat, and 55 per cent. when the rays were received edgewise. In this last experiment *no* difference was observed in the fertility of the eggs between those nearest to the tube and those more sheltered from the direct action of the rays. From the observations recorded in (C) it is possible, and from those in (D) it is probable, that X-rays received on the side have a less destructive effect on the eggs of silkworms than those received on the flat.

(B)--Eggs of Insects exposed as Larvæ to Moderate Doses of Radiations.

(a) X-rays.

(1) **Both Parents.** — On June 17th, 1909, three lots of 24 larvæ of an average age of about a fortnight were exposed to X-rays for 5, 10, and 20 min. respectively, as described on page 237 of the Ninth Cancer Report (vol. xix. of these Archives). These doses were repeated on June 28th and July 9th. Of the 24 silkworms which had had a total dose of a quarter of an hour's X-rays (E), only 14 went into the chrysalis stage, and in 2 of these moths never emerged. Seven were males and 5 females. Three pairs of these were separately mated, their eggs collected, and 200 from each series counted when hatching was complete. The mean percentage hatched from the three series was 90·5. Of the 24 silkworms which had had a total dose of half an hour's X-rays (F), 23 came to maturity, as 15 males and 8 females. Three series of eggs, each of 200, were counted from these in the same way, and gave an average fertility of 97·5 per cent. Of the 24 silkworms that had had the largest dose, 60 min. (G), 18 reached maturity ; 16 moths emerged as 6 males and 10 females. Three series of fertilised eggs were counted, the mean percentage hatched being 90 per cent. ; 90·5 of control eggs hatched out.

During 1910 the above experiments were continued. Sixty larvæ from that series (F) in which X-rays had apparently produced the greatest effect were selected and divided into two groups of 30 each. To one of them, as to the previous generations, a total exposure of 30 min. X-rays in 3 doses of 10 min. each, given at intervals of one week, was ad-

ministered. The other series had no X-rays at all. Twenty-nine of this latter series entered the pupa stage, emerging as 12 males and 16 females. One failed to emerge. The average fertility of 12 pairs of this series was 94 per cent. (maximum 97 per cent., minimum 88 per cent.) compared with 91 per cent. in the general controls. Of those caterpillars that had had X-rays applied, 25 went into the pupa stage, and emerged as 9 males and 16 females. The average fertility of 3 pairs was 82 per cent. (maximum 83 per cent., minimum 80 per cent.), compared with a fertility of 91 per cent. in the controls.

In the spring of 1910 another series of experiments was carried out. Sixty small control silkworms were selected and X-rays applied for 30 min. in 3 doses of 10 min. each, separated by a week's interval. Fifty of them went into the pupa stage, and the average fertility calculated from the eggs of 1911 was 82 per cent., compared with 91 per cent. in the controls.

The only conclusion to be drawn from these figures is that no very considerable effect on the fertility of the eggs of silkworms is to be attributed to the application of X-rays in moderate doses to the insects in the larval stage.

(2) **One Parent.**—Observations were also made upon the effect of the application of X-rays to one parent only in the caterpillar stage. Two male moths which, as caterpillars, had had a total dosage of 60 min. X-rays between June 17th and July 8th, 1909, were mated, each with two control females. The resulting eggs showed an average fertility of 93·0 per cent. (maximum 98·5 per cent., minimum 87·5 per cent.), compared with a fertility of 90·5 in the controls.

Thirty of the caterpillars from the series showing the greater fertility (98·5 per cent.) were retained in the spring of 1910. Twenty-five entered the pupa stage, and emerged as 16 males and 9 females. The average fertility of the eggs collected from 8 pairs was 68 per cent., compared with 91 per cent. in the controls. The retarding action from X-rays would appear to be observable in the second generation from this experiment.

(b) Radium.

On July 7th, 1909, eight small silkworms were selected from among the controls and placed in a lead-covered box, in

the bottom of which was a glass tube containing ·88 milligram of radium bromide. The smallest caterpillars were selected, as it was hoped that they would spin later than the others, and so they might have a longer application of radium. This was found to be the case, and with the exception of one that died, all the worms went into the chrysalis stages on July 25th to 30th. When the moths emerged on August 12th to 15th, it was found that they were all males. It is most probable that this effect is to be attributed, not to the action of the radium, but rather to the selection of the smaller caterpillars. Two of the radium males emerging on August 15th were mated with two control females emerging on the same day. The fertility of the eggs from these worked out as 93·2 per cent., compared with a fertility of 90·5 per cent. in the controls.

Some of the caterpillars which emerged from the above in 1910 had no further radium treatment, while with others the application of radium was continued. Of the 30 untreated by radium, 27 reached maturity and emerged from the chrysalis stage as 11 males and 15 females. One was lost. Eleven pairs were mated, and the fertility of their eggs worked out as 69 per cent., compared with 91 per cent. in the controls. On June 9th, 1910, 10 caterpillars of an average age of about 4 days, the parents of which had been exposed to radium in 1909 as above, were selected and enclosed in a small lead-covered tray $3\frac{1}{2}$ in. $\times 3\frac{1}{2}$ in. In the centre of the floor of the tray was fixed a small glass bulb containing ·88 mgr. radium bromide. Five of these died as caterpillars, and one just before it reached the pupa stage. Another formed an imperfect cocoon from which no moth emerged. One male and two female moths alone emerged, and the male had imperfect wings and appeared very feeble. It was mated with the females and eggs were laid, but they proved to be sterile.

On June 9th, 1910, ten control caterpillars of an average age of four days were placed in a lead-lined tray $3\frac{1}{4}$ in. $\times 3\frac{1}{4}$ in. in the floor of which was fixed a tube containing ·735 mgr. of radium bromide. Seven of these reached the chrysalis stage, but in three of them the cocoon was imperfectly formed. From three of the four remaining cocoons moths emerged. Two were females and the third was lost before its sex was determined.

Although the caterpillars selected for radium treatment in 1910 were average specimens and apparently identical with the controls, yet not only was development interfered with as seen above, but it was also much delayed and the chrysalis stage was entered about a fortnight later than was the case in the controls. (See, however, note on p. 150.)

(C)—Eggs of Insects exposed as Pupæ to Moderate Doses of Radiations.

Ten cocoons (H) of control worms spinning on July 23rd and 24th, 1909, were taken. Ten minutes X-rays were given on August 5th and August 6th (total 20 min.), without removal of the silk. The moths emerged on August 11th as five males and five females, and were immediately mated. The resulting eggs showed a fertility of 70 per cent. as compared with 90·5 per cent. in the controls. Ten cocoons (I) formed on July 25th were taken and treated similarly except that just double the dosage of X-rays was employed (total 40 min.). The moths emerged on August 11th, 12th, and 13th, as six males and four females. The resulting eggs showed a fertility of 87 per cent. as compared with 90·5 in the controls. Ten cocoons (K) spun on July 27th were given 30 min. X-rays on the same two dates (total 60 min.). The moths emerged between August 11th and August 14th, as seven males and two females. One failed to emerge. The sexes were allowed to mix, and the resulting eggs gave a fertility of 94 per cent. as compared with 90·5 in the controls.

Thirty of the caterpillars whose parents had received a total dosage of 20 min. X-rays in 1909 (H) were retained during the spring of 1910. Twenty-two entered the chrysalis stage, emerging as nine males and thirteen females. Some of them were X-rayed as cocoons for 10 min. on each of two consecutive days (total 20 min.) after temporary removal of the silk. In other words the dose received in the previous generation was repeated. Four pairs were thus treated, and the average fertility of the resulting eggs proved to be 67 per cent. (maximum 70 per cent., minimum 63 per cent.) as compared with a fertility of 91 per cent. in the controls. Besides the above other chrysalids, after temporary removal of the silk casing, were exposed for 10 min. on each of six consecutive

days (total 60 min.). Two pairs were thus treated and gave an average fertility of 54 per cent. compared with 91 per cent. in the controls. Three males from the above which had received two 10 min. doses of X-rays in the cocoon stage were mated with three females which had received six such doses in this stage, and 67 per cent. of the eggs hatched out.

Again thirty of the caterpillars whose parents had received a total dosage of 60 min. X-rays in 1909 (K) were retained in the spring of 1910. Twenty-six of them entered the pupa stage and emerged as nineteen males and seven females. As chrysalids some received X-rays for 10 min. daily after removal of the silk on each of six consecutive days (total 60 min.). Others received X-rays for 10 min. on two consecutive days (total 20 min.). One pair received no X-rays at all in this generation. Three pairs which had had X-rays for 60 min. gave an average fertility of 42 per cent. Three pairs which had received the X-rays for 20 min. showed 59 per cent. of fertile eggs. The single pair which had had no X-rays at all gave a fertility of 57 per cent. as compared with 91 per cent. in the controls.

Further experiments on control chrysalids were carried out in 1910. Four pairs were taken as soon as spinning was completed and the silk removed. X-rays were applied to the pupæ for 10 min., and the dose was repeated the following day (total 20 min.) (H). The fertility of the resulting eggs was 88 per cent., compared with 91 per cent. in the controls. In three pairs which were treated exactly similarly except that X-rays were applied for 10 min. on each of six consecutive days (total 60 min.) (K) the fertility was 63 per cent.

From the above observations it would appear that the effect of the application of X-rays in the pupal stage on the fertility of silkworms' eggs is most marked in the second generation.

(D)—Eggs of Insects exposed as Moths to Moderate Doses of Radiations.

The effects of the application of X-rays to moths before mating were also observed. Five control male and four

control female moths (L) emerging from the cocoons on August 7th, 1909, were taken and X-rays applied to each for 10 min. On each of the two following days the dose was repeated (total 30 min.), and the moths then mated. Of the resulting eggs 92 per cent. were fertile, the fertility of the controls being 90·5. [Cp. (Q), *infra*.] To four male and four female control moths (M) emerging on August 11th a single dose of 40 min. X-rays was administered before mating. The fertility of the resulting eggs was 76 per cent. Four male and three female control moths (N) emerging on August 12th were kept separate until 30 min. X-rays had been administered to them on that day and on each of the next three following (total 120 min.). The fertility of the resulting eggs proved to be 95·5 per cent., the controls being 90·5.

In the summer of 1910 the above observations were continued. Thirty of the caterpillars whose parents had received a total dosage of 30 min. X-rays (O) were retained. Twenty-eight entered the chrysalis stage, emerging as 11 male and 16 female moths. Four pairs of these had no further treatment, but were simply mated; 86 per cent. of their eggs were fertile, compared with 91 per cent. in the controls. Three other pairs had the treatment meted out to their parents in 1909 repeated; that is, X-rays were applied for 10 min. on three consecutive days before mating; 89 per cent. of their eggs were fertile. Three other pairs were given a single exposure of X-rays for 10 min. and were then immediately mated; 83 per cent. were fertile.

In the same way thirty of the caterpillars whose parents had received a single dose of 40 min. X-rays (P) in 1909 were retained in 1910. Twenty-six went into the chrysalis stage and emerged as 12 males and 14 females. Five pairs were mated and gave a fertility of 63 per cent., compared with 91 per cent. in the controls. Five other pairs were X-rayed as moths for 40 min. and then immediately mated, the treatment of 1909 being repeated; 60 per cent. of the eggs were fertile.

Two pairs of control moths which emerged from cocoons on July 29th, 1910, had 30 min. X-rays administered immediately before mating. The fertility of the resulting eggs

was 69 per cent., compared with 91 per cent. in the controls. In the case of two pairs that were given a single exposure of 40 min. to the rays 85 per cent. of the eggs were fertile. Where X-rays were administered for 10 min. a day on three consecutive days before mating (Q) two pairs, the average fertility was 70 per cent. [Cp. (L), *supra*.]

E).—Eggs of Insects exposed in more than one stage of development to Moderate Doses of Radiations.

(1) **Both Parents.**—Some cocoons which, in the caterpillar stage, had been exposed to X-rays for 10 min. every

TABLE I.

GENERAL TABLE OF RESULTS AS REGARDS FERTILITY.

Stage in which Radiations were applied.	Treatment in 1909.	Percentage of eggs hatched in 1910.	Treatment in 1910.	Percentage of eggs hatched in 1911.
CONTROLS	No X-rays ...	90·5	No X-rays ...	91
LARVÆ—Both parents	15 minutes X-rays	90·5	... ...	—
,, ,,	30 ,, ,,	97·5	30 minutes X-rays	82
,, ,,	30 ,, ,,	,,	No X-rays	94
,, ,,	60 ,, ,,	90·0	... ...	—
,, ,,	No X-rays (control)	90·5	30 minutes X-rays	82
One parent...	60 minutes X-rays	93·0	No X-rays ...	68
,, ,, ...	7 weeks radium...	93·2	No radium ...	69
PUPÆ—Both parents...	20 minutes X-rays	70	20 minutes X-rays	67
,, ,,	20 ,, ,,	,,	60 ,, ,,	54
,, ,,	40 ,, ,,	87	... ...	—
,, ,,	60 ,, ,,	94	60 minutes X-rays	42
,, ,,	60 ,, ,,	,,	20 ,, ,,	59
,, ,,	60 ,, ,,	,,	No X-rays ...	57
,, ,,	No X-rays (control)	90·5	20 minutes X-rays	88
,, ,,	,, ,, ,,	,,	60 ,, ,,	63
MOTHS—Both parents	30 minutes X-rays	92	30 minutes X-rays	89
,, ,,	30 ,, ,,	,,	10 ,, ,,	83
,, ,,	30 ,, ,,	,,	No X-rays ...	86
,, ,,	40 ,, ,, *	76	40 minutes X-rays*	60
,, ,,	40 ,, ,, *	,,	No X-rays ...	63
,, ,,	120 ,, ,,	95·5	... ...	—
,, ,,	No X-rays (control)	90·5	30 minutes X-rays*	69
,, ,,	,, ,, ,,	,,	40 ,, ,, *	85
,, ,,	,, ,, ,,	,,	30 ,, ,,	70

* X-rays in these cases were administered for the time indicated as a single dose, in all others in several doses as stated in text.

week for three weeks (total 30 min.) in 1910, and also in the previous generation, 1909, were treated by X-rays for 10 min. on each of the succeeding days after the removal of the silk (total 40 min.). When a pair of moths emerged they were immediately mated, and the resulting eggs gave a fertility of 47 per cent. compared with 82 per cent. in the untreated cocoons and 91 per cent. in the controls. Another male moth from these cocoons was mated with a female whose parents had in 1909 received X-rays for a total of one hour in the cocoon stage, and which had itself been given X-rays for 10 min. in the cocoon stage on two occasions. Before mating each moth was exposed to X-rays for 3 hours. Only 9 per cent. of the resulting eggs hatched, compared with 82 per cent. in the untreated cocoons and 91 per cent. in the controls.

In another experiment cocoons from the same series, i.e. those derived from caterpillars which had received a total dosage of 30 min. X-rays in both generations (1909 and 1910)

TABLE II.—THE VALUES OF TABLE I. RE-ARRANGED, AND IN EACH CASE CORRECTED TO THE APPROPRIATE CONTROL AS 100.

Stage in which X-rays were applied.	Total Exposure to X-rays (minutes).	Fertility of 1st Generation (X-rayed).	Fertility of 2nd Generation (not X-rayed).	Fertility of 2nd Generation (X-rayed as in 1st).
LARVÆ—Both parents ...	15	100	—	—
" "	30	108	103	87
" "	30	90	—	—
" "	60	100	—	—
One parent ...	60	103	75	—
PUPÆ—Both parents ...	20	77	—	—
" " ...	20	97	—	—
" " ...	40	96	—	—
" " ...	60	104	63	74
" " ...	60	69	—	—
MOTHS—Both parents ...	30	102	95	104
" " ...	30	76	—	—
" " ...	30	77	—	—
" " ...	40	84	69	95
" " ...	40	93	—	—
" " ...	120	106	—	—

were taken. The male cocoon had X-rays for 10 min. a day for 9 successive days. The female had X-rays for 10 min. for 7 successive days as a chrysalid and 10 min. a day for 2 days as a moth. Directly the male emerged mating took place, and 50 per cent. of the resulting eggs proved fertile.

(2) **One Parent only.**—In order to try the effect of X-rays on one parent only, an untreated male control was mated with a female which had had X-rays for 10 min. on successive days, 5 times in the pupa stage and once as a moth; 73 per cent. hatched, compared with 82 per cent. in the controls. And a similar male control was mated to a female which had had seven of these doses as a pupa and two as a moth; 46 per cent. hatched, compared with 82 per cent. in the controls.

The results of this part of the investigation are summed up on p. 151, and are shown in tabular form on pp. 138–9.

II.—EFFECT OF MODERATE DOSES OF RADIATION ON THE DATE OF HATCHING OF EGGS.

Two series of observations were made in the summers of 1910 and 1911 respectively, and the results are recorded here.

(i) The first series continues and extends that already published in the Ninth Volume of these Cancer Reports above referred to. The full experimental details there given are very briefly summarised in the first four columns of the table given below. The first column contains the number of eggs observed in each series of experiments; the second indicates the nature of the radiations applied and the stage of development attained by the silkworms at the time of application; the third and fourth columns show respectively the number of exposures to radiation and the total duration of exposure in each case, while the fifth shows the percentages of eggs hatched out by June 7th, 1910. A month later, July 6th, all or practically all the larvæ which were capable of emerging from the eggs had done so, and the percentages of eggs hatched out, counted on that date, are given in the sixth column as the total percentages hatched; the seventh column shows, also in the form of percentages, the ratios of the percentages given in the fifth column to those contained in

the sixth, and thus indicates roughly any acceleration or retardation in date of hatching out of the eggs to be observed in the various cases.

No. of eggs observed.	Character of Experiment.	No. of Exposures.	Total Exposure (Minutes).	Percentage hatched by 7/6/10.	Total Percentage hatched.	Percentage of Total Hatching by 7/6/10.
1,000	Control	0	0	50·4	90·5	55·6
600	Both parents irradiated as caterpillars (X-rays)	3	15	65	90·5	71·8
600		3	30	85	97·5	87·2
600		3	60	55	90	61·1
400	Male parent irradiated as caterpillar: (*a*) X-rays	3	60	76	93	81·7
200	(*b*) Radium throughout caterpillar stage	—	—	66	93·5	70·6
200	Both parents irradiated as chrysalids	2	20	42	70	60·0
200		4	40	53·5	87	61·5
200		6	60	67·5	94	71·5
200	Both parents irradiated as moths	3	30	88·5	92	95·6
200		1	40	57·5	76	75·6
200		4	120	59	95·5	61·8
200	Eggs irradiated shortly after laying	3	45	76	95	80·0
200		4	60	59·5	84	70·8

On examination of the foregoing table a most striking fact is at once observed, viz., that in every case after irradiation, whether of both parents or one only, the percentage of eggs hatched out during the early period is increased as compared with that found in the case of the controls. A similar result is found even when not the parents, but the eggs themselves, have been irradiated shortly after laying. (As connecting the two generations in this respect more closely than is evident at first sight, it may be remarked here, in parenthesis, that irradiation of silkworm moths, or even earlier forms, might to some extent be regarded as irradiation of reproductive cells; for example, a female moth might be

regarded in some degree as a bag of unfertilised ova.) Thus it would appear that—

(1) X-radiation employed as above accelerates the hatching of silkworm ova.

(2) Such acceleration takes place irrespective of the stage of life-history at which irradiation is performed—whether on the eggs themselves soon after they are laid, or on their parents (one or both) as moths, chrysalids, or larvæ.

It is evident that the number found for the control in the above table (viz., 55·6) is of primary importance, and that liability to experimental error must be carefully considered. Where several series of eggs were used in the different experiments, the mean results are given in this table, which is completed below by a further table giving the maximum and minimum percentages for the separate series, thus :—

—	No. of Series of 200 Eggs for Experiment.	Maximum Percentage Hatched 7/6/1910.	Minimum Percentage Hatched 7/6/1910.	Maximum Percentage Hatched 6/7/1910.	Minimum Percentage Hatched 6/7/1910.
Control	5	74·5	35	93·5	85
Both parents X-rayed as larvæ for 15 min.	3	88·5	39	96·5	84·5
Both parents X-rayed as larvæ for 30 „	3	95·5	76	98	97
Both parents X-rayed as larvæ for 60 „	3	70	44·5	94	86·5
Male parent X-rayed as larva	2	87·5	64·5	98·5	87·5

It is thus seen that experimental error is liable to be large ; against this must be set—

(1) The fact that five series of 200 eggs, i.e. no fewer than 1,000 eggs, were used in calculating the control figure ;

(2) The fact that the difference between the control figure (55·6) and the average figure (73·5) obtained from the remaining percentages in the last column of the previous table is very considerable, and allows for a large margin of experimental error ;

(3) The further evidence incidentally furnished by the following series of experiments in which separate and independent controls were of necessity used in the several experiments, each set of which supplies a result in accordance with the foregoing conclusions.

(ii) The effect of X-ray treatment of silkworms on the time of hatching out of their progeny has been observed for a second year in each of four groups of cases with independent controls, the test applied being again the percentage of the total hatching in each case occurring before the date June 6th, 1911, and the special object aimed at being information as to the effect of repetition of X-ray treatment in a second generation of silkworms. In two of these four groups the X-rayed silkworms were themselves the progeny of silkworms treated with X-rays, while in the other two they were the offspring of silkworms which had not been so treated. The experimental details are indicated in the following table :—

Treatment in 1909.	Number of Exposures.	Total Exposure (Minutes).	Treatment in 1910.	Number of Exposures.	Total Exposure (Minutes).	Percentage of Total Hatching by 6/6/1911.
(1) Nil	0	0	X-rayed as Larvæ	3	30	30
			X-rayed as Pupæ	6	60	71
			X-rayed as Pupæ	6	60	85
			Nil (control)	0	0	22
(2) Nil	0	0	X-rayed as Pupæ	6	60	53
			Nil (control)	0	0	16
(3) X-rayed as Pupæ	6	60	X-rayed as Pupæ	6	60	19
			Nil (control)	0	0	2
(4) X-rayed as Moths	1	40	X-rayed as Moths	1	40	20
			Nil (control)	0	0	12

(1) In the first series of experiments it is observed that an acceleration in date of hatching out is shown by eggs whose parents have been X-rayed either as larvæ or pupæ, moderate in the former case, very great in the latter.

(2) In the second series a great acceleration is again observed in the case of eggs whose parents have been X-rayed as pupæ.

In these two series it will be noted that the control numbers 22 and 16 are themselves small as compared with the general control number 55·6 of the previous year; the average

is 19, indicating that in the case of eggs which had never been treated by X-rays in any previous generation about one-fifth only of the total hatching was completed by June 6th in 1911.

(3) In the third series several interesting points are observed. In the first place, disregarding remoter ancestry, it is once more seen that marked acceleration in date of hatching out is shown by eggs whose parents have been X-rayed as compared with eggs whose parents have not been so treated. But, taking this remoter ancestry—the grandparentage—into account, two other points emerge. The control number 2 is not only small in itself, but it is also small as compared with the control numbers and their average in Series (1) and (2) of experiments; in other words, irradiation of grandparents and not of parents has resulted in retardation in date of hatching out. Finally, it is observed that the percentage of early hatching out in this series after irradiation in two successive generations is 19—i.e. about one-fifth of the total hatching was done by June 6th, just as in the case of the controls of Series (1) and (2), which had never been treated by X-rays in any previous generation, thus indicating that retardation due to irradiation of grandparents in 1909 had been neutralised by acceleration due to irradiation of parents.

(4) In the fourth series of experiments every point indicated in the preceding paragraph (3) receives confirmation.

III.—EFFECT OF MODERATE DOSES OF RADIATION ON THE WEIGHT OF COCOONS.

During the summer of 1910 an extended series of observations was made on worms hatched out from those sets of eggs, laid during 1909, in which the most marked effects of acceleration or retardation had been produced by radium or X-rays, and an attempt was made to increase the action by repeating the same treatment in a second generation.

Thirty silkworms of each of 11 different series (including two controls), all of about the same size and of an average age of perhaps a week, were selected and fed on mulberry leaves. Each of these series was carefully weighed in the pupa stage,

while in the cases of the two control series each individual cocoon was weighed and its sex afterwards determined so as to obtain the relative weights of males and females. As the mean of the 59 controls (one failed to reach maturity) it was found that the average weight of a male cocoon was 1·062, and of a female 1·460 grm.

The results are best shown in a table, the fourth column of figures in which gives the calculated weight in grammes of 10 males and 10 females in each series, so that these figures are directly comparable with each other.

—	—	Number in series weighed	Weight in grammes.	Weight of 10 males + 10 females.	Result as compared with control.	Average result of all series.
Controls ...	—	59	75·72	25·22	—	—
X-rays as Larvæ	Both parents (30′) and offspring (30′)...	25	33·2	24·44	— ·78	Increase (+ ·40)
	Both parents only (30′)	29	38·9	27·12	+ 1·90	
	Male parent only (60′)	25	30·9	25·85	+ ·63	
	Offspring only (60′) ...	50	65·65	25·08	— ·14	
X-rays as Pupæ	Both parents only (20′)	22	27·74	24·52	— ·70	Increase (+ ·77)
	„ „ (60′)	26	33·1	27·46	+ 2·24	
X-rays as Moths	Both parents only (30′)	28	37·4	25·97	+ ·75	Increase (+ ·94)
	„ „ (40′)	26	34·4	26·35	+ 1·13	
X-rays as Eggs	A few days after laid...	29	42·8	27·01	+ 1·79	Increase (+1·79)
Radium as Larvæ	Male parent only ...	27	34·49	24·95	— ·26	Decrease (—5·31)
	Male parent only and offspring	4	2·85	14·25	—10·97	
	Offspring only... ...	7	5·86	20·51	— 4·71	

In comparing these figures of 1910 with those of 1909 (loc. cit., p. 238) it will be at once seen that the results obtained are less constant. In 1909 a considerable increase was found in the weights of cocoons resulting from larvæ treated by X-rays. This result may perhaps be, in part at any rate, explained by the fact that the larvæ for these experiments were obtained with considerable difficulty late in the season.

when the caterpillars hatched out are on the whole markedly smaller and less robust than those obtained earlier, many never arriving at maturity.

It is to be noted that the significance of the whole series of figures hangs upon the control, which is therefore of primary importance. (Cf. p. 142.) Taking this as accurate, however, it will be quite evident that, as employed above *in the first generation*, X-rays have a stimulating effect on larval growth. The weight of the cocoons is increased in six series of experiments and diminished in three; but not only is the former number twice as great as the latter, but the average increase (+ 1·41) is nearly three times as great as the average decrease (− 0·54).

From the table it is also seen that radium as used in the present series of experiments exerts only a depressing effect on larval growth.

Two sets of experiments were carried out in order to find the effect produced by the repetition of X-ray treatment *in the second generation* of silkworms.

(1) In the first series of experiments, chrysalids were irradiated on August 5th, 1909, for 10 min. as described in the first section of this paper, and the treatment was repeated on August 6th, 7th, 8th, 9th, and 10th, giving a total exposure of 60 min. Eggs (A) resulting from the mating of moths emerging from cocoons so treated gave rise to chrysalids which were in turn irradiated for 10 min. on July 8th, 9th, 10th, 12th, 13th, and 14th, 1910, the total exposure being again 60 min., while other eggs (B) were allowed to develop without treatment as controls. The eggs resulting from the two sets of moths emerging from experimental and control cocoons were then allowed to develop without further treatment, and it was found that of the cocoons thus obtained the experimental were markedly lighter than the control, the former, 16 in number, averaging ·557 grm. each, while the latter, 14 in number, averaged ·711 grm. each, the corrected average weights working out as—

(A) Experimental ... F. ·624 grm., M. ·446 grm.
(B) Control ... ... F. ·809 ,, M. ·578 ,,

(2) In the second series of experiments moths were irradiated on August 12th, 1909, with a single exposure of

40 min. Eggs (A) resulting from the mating of these irradiated moths gave rise to moths which were in turn irradiated with a single exposure of 40 min. on July 25th, 1910, while other eggs (B) were allowed to develop without treatment as controls. The eggs resulting from experimental and control moths were then allowed to develop without treatment, and it was found that although the resulting 26 experimental cocoons were slightly heavier on an average than the 12 control, the former averaging ·612 grm. each and the latter ·589 grm., yet this was due simply to the great preponderance of females in the latter case (10 females to 2 males as against 15 females to 11 males in the former), for the corrected average weights worked out as—

(A) Experimental ... F. ·643 grm., M. ·460 grm.
(B) Control ... ... F. ·670 grm., M. ·479 grm.

The two sets of results are thus in agreement with one another, and may be tabulated thus:—

Treatment in 1909.	Treatment in 1910.	Weight of F. cocoon.	Weight of M. cocoon.
(1) Irradiated as pupae (6 exposures of 10 min. each)	A. Irradiated as pupæ (6 exposures of 10 min. each)	·624 grm.	·446 grm.
	B. Nil (control) ...	·809 grm.	·578 grm.
(2) Irradiated as moths (1 exposure of 40 min.)	A. Irradiated as moths (1 exposure of 40 min.)	·643 grm.	·460 grm.
	B. Nil (control) ...	·670 grm.	·479 grm.

It is at once evident from the table that, as far as these experiments go, repetition of X-ray treatment in the *second* generation produces a *depressing* effect, as regards weight, on larval growth.

IV.—EFFECT OF LARGE DOSES OF RADIATIONS, WHEN APPLIED TO LARVÆ, ON WEIGHT AND VITALITY.

The following experiments were performed in the hope of obtaining further evidence of a stimulating effect of X-rays upon the growth of silkworms, and of the effect produced by a continuous exposure to radium. More frequent

exposures to X-rays were given, and the worms were weighed at intervals, and their weights compared with those of an equal number of controls. During exposure the worms were placed in a Petri dish without leaves, and the control worms were also deprived of food for a similar time.

(a)—X-rays.

(1) On June 2nd, 1911, 24 silkworms 3 to 6 days old were divided into 2 lots. One lot of 12 worms was exposed to X-rays for half an hour in a Petri dish covered with muslin which was just touched by the bulb. The spark-gap was 9·3 cm., and the current through the primary 4·5 amperes. Under these conditions there was a marked rise of temperature in the dish; for the later exposures, therefore, the dish was placed with its muslin cover 1·8 cm. below the bulb, the rise of temperature under these conditions being but slight. Similar exposures were made on June 6th, 12th, 15th, 19th, 22nd, 26th, and 29th, and on July 3rd.

June 23rd.—Controls appear if anything slightly larger than exposed worms, but there is no marked difference.

June 26th.—Controls definitely larger than exposed worms.

June 27th.—Average weight of exposed 12 worms is ·138 grm., and of the 10 control worms still alive ·252 grm. After the exposure on July 3rd the exposed worms appeared very ill, making ineffectual efforts to change their skin, and were much smaller than the control worms, which appeared healthy. All the exposed worms died within the following week. At no time was a stimulating effect observed.

(2) On June 6th, 24 worms 5 to 6 days old were divided into 2 lots, and 12 worms exposed for 1 hour every day except Sunday, the muslin cover being 1·8 cm. below the bulb. During the first five exposures there was a considerable rise of temperature (as high as 29° C.) in the dish; in the later experiments with a new bulb rise of temperature was trifling. Spark-gap 7–11 cm.; current ·5 ampere.

June 17th.—Exposed worms appear smaller than controls.

June 23rd.—Exposed worms appear much smaller than controls, and very torpid. Exposures stopped.

June 28th.—Exposed worms all dead; control worms all alive and well developed.

(3) Older Worms.—On June 21st, 40 worms 12–13 days old were divided into two lots, and 20 worms exposed to X-rays as in (2), the exposure being repeated on June 23rd, 26th, 28th, and 30th, and on July 3rd.

June 28th.—Exposed worms, if anything, slightly smaller than controls.

July 4th.—Of the exposed worms 8 are dead and 10 look ill, while 19 control worms are healthy.

July 13th.—Exposed worms all dead.

The foregoing experiments having demonstrated that the doses given were highly injurious, further experiments were performed with shorter exposures; and older worms were used.

(4) On June 29th, 18 worms 22 days old were divided into 2 batches and weighed, and one batch exposed for 10 min. to X-rays 3 days per week. The average weights were as follows:—

June 29th.—Radiated (9 worms) ·0172 grm., control (9 worms) ·0167 grm.

July 12th.—Radiated (8 alive) ·1493 grm., control (8 alive) ·1450 grm.

July 20th.—Radiated (8 alive) ·376 grm., control (8 alive) ·395 grm.

The worms commenced spinning about July 31st; the last exposure to X-rays was made on July 29th.

Average weights of cocoons: Radiated (7 in number) ·503 grm.; control (8 in number) ·562 grm.

At no time was any stimulating effect apparent; there was a decided decrease in weight, though not so marked as in the previous experiments; moreover, while of the control worms 5 healthy males and 3 females (2 ill-developed) emerged from the cocoons, of the radiated only 2 males and 1 female, all ill-developed, emerged; 4 never emerged at all, and the proportion of males to females could not be ascertained.

(5) On June 29th, 28 worms three weeks old were divided into two sets and one of these exposed to soft rays two days per week. The average weights were as follows:—

June 29th.—Radiated (14 worms) ·0101 grm.; control (14 worms) ·0100 grm.

July 12*th.*—Radiated (14 alive) ·0691 grm.; control (13 alive) ·0637 grm.

July 20*th.*—Radiated (14 alive) ·2629 grm.; control (13 alive) ·2519 grm.

So far there is little difference in the weights, the radiated being very slightly heavier than the controls; but when spinning commenced (about August 4th) the control worms were considerably larger than the radiated, and a deleterious effect was shown on emergence of the moths.

Average weights of cocoons: Radiated (13 in number) ·475 grm.; control (12 in number) ·638 grm.

From the control cocoons 5 males and 7 females emerged; from the radiated emerged 6 ill-developed males, 1 female, 2 ill-developed moths of doubtful sex, while 4 never emerged.

(b)—Radium.

(1) On June 20th silkworms 3 days old were placed in a box together with 13 milligrams of radium contained in a glass tube, while 20 worms of the same age were placed in another box as controls.

The worms with the radium did not flourish, and were all dead by June 28th, all the controls remaining alive and healthy.

(2) On June 26th, 12 worms 17 to 18 days old were placed with radium as in (1), and 12 similar worms kept as controls. On July 7th the radiated worms appeared unwell; 11 were alive and of average weight ·028 grm., while the 12 controls averaged ·112 grm. The radium was removed, but all the radiated worms died within a few days.

Note on Effect of Radiations on Time spent in Pupal State.

The average time spent in the pupal state by the 59 control cocoons (p. 145) was 23·55 days. In the case of those treated by radium the time was reduced to 19·6 days; otherwise the time was fairly constant—in only one series (those whose parents were X-rayed for 20 min. in 1909) did the mean time spent in the pupal stage fall below 23 days, and in no case did it reach 24 days.

Other Points of General Interest.

(1) A moderate inequality in the numbers of the sexes mated seems to have very little effect on the fertility of the resulting eggs. Thus 8 females were mated with 1 male and yet 69 per cent. of the eggs were fertile as compared with 55·6 per cent. in the controls. Again, 4 females were mated with 1 male, and 76 per cent. of the eggs hatched out as compared with 82 per cent. in the controls (Cf. p. 129).

(2) Where, however, the male has been previously mated, there is a definite decrease in fertility. Thus 4 females were mated with 2 males which had themselves been 9 days previously mated with other females, and only 31 per cent. of the resulting eggs were fertile as compared with 82 per cent. in the control. Again, 5 females were mated with 2 males previously mated, and 63 per cent. of the eggs hatched out as compared with 91 per cent. in the controls.

(3) Some deficiency in the fertility of the eggs would also appear to result when some days are allowed to elapse between the emergence of the moths from the cocoons and their mating. Thus a pair were kept separated for 4 days before being mated, when only 31 per cent. of the eggs hatched out as compared with 91 per cent. in the controls. Again, after 6 days' separation, 1 male and 2 females produced eggs 76 per cent. of which were fertile as compared with 82 per cent. in the controls (Cf., however, p. 137).

SUMMARY OF RESULTS.

I. **Fertility of Eggs.**—In the case of X-rays applied to the insects in the first generation no definite result is obtained, though there is an indication that the fertility of these insects is somewhat diminished. The immediate descendants of these insects, however, though not themselves receiving X-rays, are markedly less fertile. If X-rays be applied in two successive generations the fertility of the second does not depart so greatly from the normal; but, nevertheless appears to be diminished.

II. **Date of Hatching out of Eggs.**—In every case in a series of 13 sets of experiments acceleration, as estimated

by the total proportion of hatching out occurring before a given date, is indicated in the case of eggs themselves subjected to X-ray treatment or the offspring of X-rayed parents; and this finding is confirmed by the results of a series of experiments made or completed during the following year. On the other hand, a retardation is indicated in the hatching out of the eggs of the second generation; while there is also some evidence that this retardation can be warded off by repetition of irradiation in the second generation.

III. **Weight of Cocoons.**—An extensive series of experiments indicates that X-ray treatment as above applied, in one generation only, stimulates larval growth, as estimated by weight of the cocoons. (Note, however, the remark as to the control figure on p. 146.) As might be expected, not every experiment shows increase in weight of resulting cocoons as compared with controls, since experimental error may in an individual case quite well mask the individual result; but on the whole the results show well-marked agreement. On the other hand, radium in the dosage employed has exerted a depressing effect as regards weight of cocoons, while there is also evidence that X-ray treatment, when repeated as above in a second generation, has a depressing influence on metabolism as judged by this criterion.

IV. **Large Doses of Radiations.**—Both X-rays and radium applied as above in large doses to larvæ exert injurious and even destructive effects; there is in this series of experiments no evidence whatever of stimulation.

Conclusion.

As regards **Radium,** the results of the experiments described in the foregoing indicate in the main inhibitory or destructive effects. Duration of the pupæ stage, however, appears to be shortened.

There is much evidence that **X-rays,** applied to silkworms in the various ways and doses stated above, can produce stimulation or acceleration of metabolic processes as judged by several standards, notably by earlier hatching out of eggs as compared with controls. There is also considerable evidence that these effects are replaced by effects of a reverse

order in the next generation, though it is possible that this reversal may be prevented by repetition of treatment.

Thus it appears that stimulation or acceleration of metabolic processes may be produced by X-ray treatment carried out within certain limits, but that these are eventually succeeded by depression and retardation, while only the latter effects are manifested when the treatment is pushed beyond these limits. Large doses of X-rays and large doses of radium have similar deleterious results.

ON THE ACTION OF THE SECONDARY X-RAYS FROM COPPER ON THE DEVELOPMENT OF THE OVA OF ASCARIS MEGALOCEPHALA.

By SOMERVILLE HASTINGS.

In the Eighth Report of the Cancer Research Laboratories (Archives of Middlesex Hospital, vol. xv.) experiments were described by Drs. Bonney and Lazarus-Barlow on the action of X-rays and radio-active substances upon the ova of *Ascaris megalocephala*, which tend to indicate that these forces have, at any rate in some cases, a dual action on the development of the eggs, appearing sometimes to stimulate and sometimes to retard this development. My own observations on the action of various chemical substances on these same ova (Ninth Report of Cancer Research Laboratories of Middlesex Hospital) indicate clearly to what a marked degree their development can be influenced by extraneous forces.

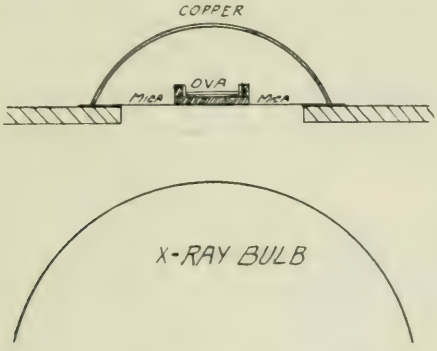
It is well known that the radiations emitted from a Crookes's tube are composite in character. Professor Barkla's* observation that when X-rays impinge upon a metallic surface the greater part of the rays given out by that surface are homogeneous and of a penetrating power dependent upon the metal used, has rendered it possible to investigate the action of X-rays upon animal cells under simpler conditions.

In the present research, therefore, an attempt has been made to determine what action (if any) the secondary radiations from copper have on the development of ova of *Ascaris megalocephala*.

Method.—Early experiments showed that an exposure for three hours to secondary rays from a plane metallic surface was not sufficient to produce changes outside the range of experimental error. It became evident, therefore, that if any

* "Brit. Med. Journal," 1910, vol. ii. (Aug. 27), p. 512.

result was to be obtained, either longer exposures must be given or some device arranged for increasing the concentration of the reflected rays. With this object in view, hemispherical surfaces of lead, iron, and copper of 8 cm. diameter on their concave surface were arranged to receive the impinging X-rays and shower the secondary rays on ova disposed as nearly as possible at the centre of the concavity. The ova spread on coverslips and covered with a very thin layer of gelatine were sheltered by thick lead from the X-rays emitted by the tube. Desiccation was avoided by frequent spraying with distilled water or by the insertion between the Crookes's tube and the metal dome of a thin sheet of mica. Temperature effects were excluded by (1) placing the control ova



obtained from the same segment of the oviduct beneath the experimental, and sheltered from secondary rays by lead, or (2) by half covering the coverslip on which the ova were spread by a piece of lead 3 mm. thick, the ova beneath this lead cover serving as the control. The actual details of the experiment can be seen by reference to the figure. In practically every case a minimum of 500 ova was counted at each observation in both experiment and control.

Results.—Five experiments were carried out with the copper dome. In four of these the exposure was for two and a half hours, and in one for one hour only. Twenty-five observations, in which experimental and control ova were counted, were made. In twenty-three of these a retardation was observed, and in two an acceleration, but in one of these the increase in the rate of development was so slight as to be

practically negligible. It may therefore be concluded that the main action of the secondary rays from copper is to retard the development of the ova.

The results of a typical experiment in which the secondary rays from copper were applied to ova for two and a half hours are recorded below.

		Single Cells.	*Two Cells.*	*Three and Four Cells.*	*More than Four Cells.*
Second Day—	Experiment	460	40	—	—
	Control	403	97	—	—
Third Day—	Experiment	185	315	—	—
	Control	96	404	—	—
Fourth Day—	Experiment	50	450	—	—
	Control	27	473	—	—
Fifth Day—	Experiment	10	487	3	—
	Control	10	453	37	—
Sixth Day—	Experiment	7	459	34	—
	Control	3	325	172	—
Seventh Day—	Experiment	7	234	259	—
	Control	5	109	386	—
Ninth Day—	Experiment	1	39	460	—
	Control	3	42	455	—

		Less than 1½ as long as broad.	*More than 1½ as long as broad.*	
Twentieth Day—	Experiment	455	45	—
	Control	433	67	—
Twenty-first Day—	Experiment	418	82	—
	Control	412	88	—

		Less than half curved.	*More than half curved.*	*Fully curved.*
Twenty-sixth Day—	Experiment	452	44	4
	Control	413	64	23

		Incomplete Circles.	*Complete Circles.*	*Living Worms.*
Twenty-seventh Day—	Experiment	425	75	0
	Control	419	75	6

Researches were also carried out, using a dome of iron instead of one of copper. Three separate experiments were made, giving an exposure of three hours in two cases and of two hours only in the third. A slight retardation was noted in 7 of the 14 observations, a slight acceleration in 3, and in the remaining 4 experiment and control were practically equal. It would appear that the secondary rays from iron have a much less marked effect on the development of the eggs of *Ascaris megalocephala* than those from copper.

FURTHER NOTES ON SUBMUCOUS TUMOURS OF THE SMALL INTESTINE.

By B. H. WEDD.

In the last volume of these Reports an account was published of a case of multiple sub-mucous tumours of the small intestine, found at the post-mortem examination of a man who had died from squamous carcinoma of the larynx. The histological appearance of these tumours differed so markedly from that in the larynx that it seemed impossible they could be metastases. One of them from which sections were made was found to infiltrate the muscular coat of the intestine, and as there seemed no reason to regard any one of them in particular as a primary malignant growth, the conclusion arrived at was that they were multiple adeno-carcinomata or endotheliomata.

When this account was published no description of exactly similar tumours had been discovered, but since then the writer's attention has been drawn to accounts of a number of cases which seem to be of the same nature.

During the past year a careful search has been made for similar tumours at post-mortems upon cancer cases. Though no fresh case of multiple tumours has been met with, single tumours have on two occasions been found, the situation and histological appearance of which were identical with those of the tumours in the case previously published.

The first of these occurred in a man aged seventy-two, who died in the cancer wards of this hospital. At the post-mortem a much ulcerated growth was found in the œsophagus, opposite the left bronchus, which proved to be a typical squamous carcinoma; a nodule in one kidney was the only metastasis discovered. About three feet from the ileo-cæcal valve, in the small intestine, a single sub-mucous nodule about the size of a threepenny-piece was found. The structure of this nodule was identical with that of those previously described, the muscular coat being extensively infiltrated.

The second case was that of a man aged fifty-six, who died in the surgical wards of the hospital after removal of the tongue for squamous carcinoma. The specimens were received from the post-mortem room described as secondary growths. The following is an extract from the post-mortem report: "About twelve inches from the ileo-cæcal valve was a firm mass about the size of a shilling, lying in the sub-mucous tissue, constricting the bowel, and surrounding more than half its circumference. An enlarged gland was present in the mesentery crossing the duodenum, upon which it was exerting pressure. No other secondary deposits found."

When sections were made, the structure of the intestinal tumour was found to be quite unlike that of the growth of the tongue, and identical with those previously described. Definite glandular acini were present in places, but the majority of the cells were undifferentiated. Numerous round or slightly oval nuclei, very clearly stained, were crowded closely together in alveoli which were separated by a stroma containing muscular fibres apparently derived from the muscularis mucosa. The growth was situated beneath the mucous membrane, which was infiltrated, as was also the muscular coat of the intestine. The gland, on section, proved to be almost entirely replaced by similar growth, though there was no acinous differentiation.

Nature of the growths.—Tumours resembling those described above have been recorded on several occasions, and various opinions expressed as to their nature.

Bunting (1904) (1) described a case of multiple primary carcinoma of the small intestine, and quotes six other cases, including two found by Lubarsch (1888)(2). Rolleston (1906)(3), in a paper on primary growths of the appendix, drew attention to the resemblance of certain of these tumours to the tumours described by Bunting. He states that 25 per cent. of the primary growths of the appendix are of a polyhedral or spheroidal celled type; the special characteristics of these growths in the appendix are (*a*) their benign nature, (*b*) their early age-incidence, (*c*) their resemblance to endotheliomata. He regards them as identical in nature with the intestinal tumours described by Bunting, though he draws attention to the difference in the average age-incidence. The resemblance

of such intestinal tumours to a primary growth of the appendix, discovered in a woman aged sixty during an operation for ovarian cyst, was independently observed by the writer.* It seems possible, having regard to the comparatively benign character of the tumours in both situations, that the apparent earlier age-incidence in the appendix may be accounted for by the production of symptoms leading to operation.

Oberndorfer (1907) (4), at the Deutsche Pathologische Gesellschaft, described six cases which he had collected of tumours of the small intestine which appear from his description to be of a similar nature. He described them as small, the size of a millet-seed to that of a split pea, often multiple, frequently occurring at the mesenteric attachment, but also in other situations, submucous in position, invading the mucosa but not the muscular coat of the intestine, having a stroma containing muscle fibres, and the structure of an adeno-carcinoma. The opinion of most of those present was that the tumours were of a simple nature, probably arising from pancreatic rests; there was not a general agreement on the subject, and their resemblance to true carcinomata was acknowledged.

Turnbull read a paper before the Pathological Section of the Royal Society of Medicine, 1910, which has not been published, upon benign and malignant myoadenomata of the small intestine he had met with, which are probably of the same nature.

There seems no reason to doubt that the tumours described by Bunting and Oberndorfer, and the three cases collected by the writer, are all growths of the same nature. The only apparent difference seems to be in their malignancy. The cases collected by the present writer have all been discovered at post-mortems upon cancer patients, and it is a remarkable fact that these have all occurred in cases which have died of squamous carcinoma of the upper part of the alimentary tract. Squamous carcinoma in this situation is found in less than ten per cent. of the post-mortems in this hospital, but

* While this volume was passing through the press a paper appeared by Miloslavich and Namba (Zeitschr. f. Krebsforschung, vol. xii., 1912, part i., p. 14) on "Primary Carcinoma of the Appendix," with a very full bibliography. The illustrations of two cases support the view that the intestinal tumours under discussion are histologically similar to primary carcinomata of the appendix.

no record of submucous tumours of the small intestine has been discovered in other cases.

These intestinal tumours seem to exhibit malignant characters in variable degree. Oberndorfer only describes an invasion of the mucous membrane, though the appearance of the tumours resembled that of true carcinoma. In Bunting's and our cases the muscular coat was infiltrated, and in our last case, which is of special interest, a metastasis in a mesenteric gland was present. A similar low degree of malignancy is also met with in similar tumours of the appendix. In this connection the relation of simple and malignant tumours in the large intestine is of interest. Makins, Wallace, and Sargent (1912) (5) have recently described a number of cases of multiple malignant growths of the large intestine; as many as five annular carcinomata were found in one case, and out of a series of eight cases five were associated with multiple polypi, the majority of which appeared to be of simple nature, though in two cases multiple polypoid growths, having the histological structure of columnar-celled carcinoma, were found.

Origin of the Tumours.—The opinions expressed as to the origin of these tumours vary, and their resemblance to endotheliomata is generally admitted. With the object of elucidating the question, numerous sections have been stained with muci-carmine. A definite pink staining of the contents of the lumina where these were present was obtained, and though not so deeply stained as the normal mucous membrane, this fact seems to support the view that has been expressed that they arise in Lieberkühn's follicles, rather than in lymphatic endothelium, or pancreatic rests.

REFERENCES.

(1) Bunting, "Johns Hopkins Bull.," vol. xv., p. 389 (1904).

(2) Lubarsch, "Virchow's Archiv.," Bd. cxi., s. 316 (1888).

(3) Rolleston, "Transactions Med. and Chirurg. Soc.," vol. 89 (1906).

(4) Oberndorfer, "Centralb. für Path.," vol. 18, p. 807 (1907).

(5) Makins, Wallace, and Sargent, "Proc. Royal Soc. Med.," vol. v., p. 137 (1912).

THE POTASSIUM CONTENT OF THE BLOOD OF MICE, AND THE EFFECT OF AN INCREASE ON TUMOUR DEVELOPMENT.

By CECIL PRICE-JONES.

In the Ninth Report from this laboratory (Archives Middlesex Hospital, vol. xix., 1910, p. 40), J. C. Mottram, in a paper entitled "The Sodium and Potassium Content of the Blood and Tissues in Normal and Carcinomatous Cases," concludes that "the blood of carcinomatous persons contains more potassium than the normal blood; . . . . the liver, kidneys, and spleen also have a potassium content above the normal."

G. H. A. Clowes and W. S. Frisbie, "On the relationship between the rate of growth, age, and potassium and calcium content of mouse tumours" ("American Journal of Physiology," vol. xiv., 1905, p. 173), state that "mice inoculated and fed with potassium-holding material appear to be more susceptible than those inoculated and fed with calcium material"; and, "the largest yield of tumours and the most rapid growth as represented by the unit of tumour produced per unit of mice in the unit of time, is apparently associated with an equilibrium in which both elements are present, the ratio averaging from 2 : 1 to 3 : 2 in favour of potassium."

S. P. Beebe, "The Chemistry of Malignant Growths" ("American Journal of Physiology," vol. xii., 1905, p. 167), says the presence of potassium in neoplasms "does seem to be in some way associated with their remarkable nutritional activities, of which rapid growth is one manifestation."

From the work of these and other writers the suggestion arose that by increasing the potassium content of the blood of normal mice, it might be possible to raise the susceptibility of these animals to tumour development.

To inquire into this question has been the object of the present research.

The subject is considered under three sections:—

(i) The estimation of the potassium content of the blood of normal mice.

(ii) Experiments to increase the potassium content of the blood of normal mice.

(iii) The influence of an increased potassium content of the blood of mice on their readiness to "take" after tumour inoculation.

I.—THE ESTIMATION OF POTASSIUM.

The estimation of potassium was made by the spectroscopic method employed by Mottram and fully described by him in the Eighth Volume of these Reports. Since, however, this method has been the subject of some controversy, it may be useful, without giving any details of the apparatus, to indicate its general principles and the modifications which I have reason to think are desirable to ensure greater accuracy of results.

The Standard Dilution Limit.—A standard solution was employed which contained 1·9 grm. of potassium chloride to 100 c.c. of distilled water, so that 1 c.c. of the solution contains 0·01 grm. of potassium. The capillary pipettes used throughout the work were accurately measured to deliver 0·02 c.c. of solution. For the sake of convenience I call 0·02 of the standard solution "D," and I know that D contains 0·0002 grm. of potassium; and 0·02 c.c. of D+10 c.c. of water, or D diluted 500 times, contains 0·0000004 grm. (4×10^{-7}) of potassium, and 0·02 c.c. of D + 7·5 c.c. of water contains $5{\cdot}3 \times 10^{-7}$ grm. of potassium, and 0·02 c.c. of D + 5 c.c. of water contains 8×10^{-7} grm. of potassium, and so on.

The standard dilution limit ("D" limit) was fixed at that greatest dilution of D of which 0·02 c.c., after addition of two drops of strong sulphuric acid (to convert the potassium chloride into potassium sulphate), showed a definite potassium band in the spectrum on five successive observations. I found that with higher dilutions the band was not always visible on successive observations, but that if it could be seen five times in succession it was visible at every observation.

The determination of the dilution limit involves a personal factor which is subject to frequent and considerable variations. The causes of these I have not studied, but they

seem to include such influences as the brightness or dullness of the day, and various subjective states of the observer. So that I decided that it was desirable to determine the standard dilution limit immediately after each estimation of the potassium content of a specimen of blood. Most commonly the limit corresponded to $5{\cdot}3 \times 10^{-7}$, but it was often 4×10^{-7}, and sometimes 8×10^{-7}. Intermediate dilutions of the D limit were not made, whereby possibly a closer accuracy might have been attained, though probably at a disproportionate expense of time and labour.

Treatment of the Sample of Blood.—The blood was obtained from the heart, the thorax of the mouse being opened under chloroform anæsthesia; it was collected in a previously weighed test tube, and the weight of the amount of blood obtained was determined. To this were added a few drops of strong sulphuric acid, and the mixture was heated for several minutes until apparently only sulphurous acid and steam were given off, when it was assumed that all the potassium was in the form of sulphate mixed with carbon, which latter Mottram has shown exerts no influence on the rate of vaporisation of potassium (loc. cit., vol. xv., 1909, p. 116). The mixture was allowed to cool and then made up to 2 c.c. by the addition of sulphuric acid. This solution then constitutes the sample to be tested.

The estimation of the Dilution of the Sample, and the amount of Potassium in one thousand parts of Blood. — To 0·02 c.c. of the sample I give the name "T." If T showed a definite band of potassium in the spectrum on five successive observations, and if 0·02 c.c. of any dilution of T failed to do this, then the T limit was equal to the D limit; but usually T has to be diluted with sulphuric acid until the dilution limit is reached. A detailed example will serve to explain the principle:—

0·02 c.c of (T + 0·04 c.c. sulphuric acid)	gave band on five successive trials
0·02 c.c. of (T + 0·06 c.c. ,, ,,)	,, ,,
0·02 c.c. of (T + 0·08 c.c. ,, ,,)	,, ,,
0·02 c.c. of (T + 0·10 c.c. ,, ,,)	,, ,,
0·02 c.c. of (T + 0·12 c.c. ,, ,,)	failed to give band

The D limit was now determined, and found to be 4×10^{-7}. Therefore 0·02 c.c. of (T + 0·10 c.c. sulphuric acid) contains 4×10^{-7} grm. of potassium, and the entire quantity (0·12 c.c.) of this dilution contains $\frac{4 \times 10^{-7} \times 0{\cdot}12}{0{\cdot}02}$ grm. of potassium; but the entire quantity of this dilution contains 0·02 c.c. of the sample; therefore 2 c.c. of the sample contains $\frac{4 \times 10^{-7} \times 0{\cdot}12 \times 2}{0{\cdot}02 \times 0{\cdot}02}$ grm. of potassium. The amount of blood obtained was 0·1673 grm. Therefore it is clear that 1000 c.c. of blood contains

$$\frac{4 \times 10^{-7} \times 0{\cdot}12 \times 2 \times 1000}{0{\cdot}02 \times 0{\cdot}02 \times 0{\cdot}1673} = \frac{2400}{1673} = 1{\cdot}43$$

Therefore in this specimen of blood there is at least 1·43 grm. of potassium in 1,000 parts. And inasmuch as 0·02 c.c. of (T + 0·12 c.c.) failed to show the band, we find by a similar calculation that there is not as much as 1·67 grm. of potassium per 1,000 parts of this sample of blood. Probably the mean, 1·55 grm., represents fairly closely the potassium content per 1,000 in this animal's blood.

Technical Precautions.—As far as possible the positions of the various parts of the apparatus and the conditions of each step in the operation should be identical. The platinum spoon should be cleaned with hydrochloric acid and the blowpipe before each observation. The same pipette should be used for the same specimen of blood, and care be taken that the full measure is always delivered into the spoon; the difficulty attending this is one probable source of error and cause for the varying strengths with which the potassium band is visible, especially in the higher dilutions. The spoon should be slowly heated, any tendency to bubbling and spluttering being avoided; it must not be introduced into the flame until all the liquid has evaporated. The gas pressure must be constant for any one series of estimations.

Comparison of the Results obtained by the Spectroscopic Method and those obtained by the Chemical Method.

In the literature of the subject I have found no reference to the blood of the mouse, the probable explanation being

that the total amount of blood in a mouse of 20 grm. weight is only about 1·15 c.c., and it is very difficult to obtain sufficient for a chemical estimation of the potassium.

A number of chemical estimations of the amount of potassium in the blood, serum, and blood clot in various animals has been published by Abderhalden (Zeitsch. f. Physiol. Chem., vol. 25, p. 65, 1898), some of which are included in Table I. :—

TABLE I.—PARTS OF POTASSIUM PER 1,000.

	Animal.	Whole Blood.	Serum.	Blood Cells.
Estimations of potassium by chemical method (Abderhalden)*	Pig ...	... 1· 9	0·22	4· 0
	Horse ...	... 1· 1	0·20	2· 7
	Rabbit ...	... 1· 7	0·21	4· 3
	Rabbit ...	... 1·61	—	—
	,, ...	... 1·87	0·31	3· 9
Estimations of potassium by the spectroscopic method (Author)	Mouse (normal) ...	—	0·30	3· 7
	,, ,,	... 1·09	0·47	4·04
	,, ,,	... 1·20	0·35	—
	,, ,,	... 1·47	0·45	4·42
	,, (cancer)	... 1·68	0·86	5·31
	,, ,,	... 1·96	0·84	4·34
	,, ,,	... 1·99	0·83	4·48

From these figures it would seem that there is much similarity in the type of results obtained by the spectroscopic method and those obtained by chemical processes. The table also shows that the greater part of the potassium of the blood is situated in the blood cells, and that the serum is relatively poor in potassium. The figures of the cancer mice are interesting as indicating the increase of the amount of potassium in the blood, and also, as might have been expected, that this increase is especially found in the serum. This question has, however, no immediate bearing on the subject of this paper, and will be dealt with separately on a subsequent occasion.

* The original figures were given in amounts of K_2O per 1,000, but for sake of comparison they have been converted and expressed as amounts of K per 1,000.

Results.—Samples of blood from twenty normal mice were examined, and the amounts of potassium per 1,000 determined, as shown in the subjoined table :—

TABLE II.

Mouse.	Weight.	"At least" amount of K per 1,000.	Mean.	"Not as much" amount of K per 1,000.
1	17·78	0·98	1·06	1·15
2	13·7	1·33	1·51	1·69
3	11·7	1·43	1·55	1·67
4	18·7	1·19	1·34	1·5
5	17·7	0·96	1·03	1·1
6	13·8	1·20	1·35	1·5
7	17·56	1·40	1·55	1·7
8	17·79	1·20	1·40	1·60
9	16·62	2·04	2·40	2·70
10	16·47	1·51	1·69	1·87
11	21·49	1·06	1·10	1·14
12	19·24	0·84	0·91	0·98
13	20·0	1·02	1·10	1·19
14	15·35	1·35	1·42	1·49
15	9·5	1·43	1·67	1·91
16	15·04	1·63	1·73	1·83
17	18·48	1·02	1·06	1·11
18	13·82	1·60	1·73	1·87
19	20·71	1·16	1·29	1·42
20	24·75	1·26	1·32	1·39
Average	17·55	1·28	1·41	1·53
		Excluding No. 9.	1·36	

From these figures it appears that the potassium content of the blood of normal mice varies between 0·9 and 1·7 per 1,000 (I exclude mouse 9, which seems to be exceptional), and has a mean value of about 1·4 to 1·36.

It also appears that, generally speaking, mice that weigh over 17·5 grm. have less potassium in their blood than the smaller mice; though, admittedly, even if mouse 9 be excluded, there is no invariable relation between the size of the mouse and the potassium content of its blood. From other considerations, however, it is very probable that the potassium content of the blood is greater in young mice, owing to the usually greater number of immature polychromatic red cells, which contain more nuclear affinities, and by a consensus of opinion among observers there is a greater amount of potassium in the blood of young animals.

and in animals with nucleated red cells in their blood. Relative increase of amount of potassium is also observed in concentrated blood, which is well shown in the blood of starved animals. Varying concentrations of the blood may explain some of the varying results in the preceding table.

II.—INCREASING THE POTASSIUM CONTENT OF THE BLOOD OF NORMAL MICE.

It was important to ascertain whether it was possible to increase the amount of potassium in the blood of mice by giving an increased amount of potassium in the food. Two methods were possible: giving a diet rich in potassium, or administering a definite dose of some potassium salt.

The first attempt was to feed the mice on male herring roe, the ash of which is stated to contain about thirty-three per cent. of potassium. The roes were in tins, and probably associated with some form of preservative. The roes were given mixed up with bread. The animals ate the bread, but the roe was separated and left uneaten; whether they had objection to the roe or possibly to the accompanying preservative was not ascertained, but since the ash and the total amount of potassium is really very small it did not seem worth while to pursue the diet.

Six mice were given a food consisting of ground peas, cocoa, raisins, and dried figs; they refused to eat the raisins and figs, and whether any of the cocoa was eaten was very doubtful. After twelve days they were all alive but in a poor condition. On the thirteenth day one was killed, and an examination of the blood showed that it contained at least 1·7 and not as much as 2·04 (mean 1·87) grm. of potassium per 1,000. The animal measured 77 mm. and weighed 12·25 grm., which is too low, and this together with its poor condition made me suspect that the high amount of potassium was only relative and due to a starvation concentration of the blood; the stomach and intestines seemed empty.

On the following day four mice were found dead. The survivor was killed; it appeared in better condition; the stomach and bowels contained food; but portions of the four dead mice had been eaten, which may in part account for this.

An estimation of the blood showed a potassium content of at least 0·71 and not as much as 0·8 grm. per 1,000. This mouse measured 85 mm. and weighed only 17·87 grm., about 2 grm. too little, which suggests that it had been underfed.

Six mice were fed on bread which was soaked and squeezed out with 2 per cent. solution of potassium chloride. Each day about half of the food was uneaten. After a week, one mouse was killed. Weight 29·96 grm., length 103 mm., K content of the blood at least 1·43 and not 1·51 grm. per 1,000. The animal was healthy, well nourished, and showed a slightly raised amount of K in the blood. The other mice remained well, so that the strength of the potassium solution was increased to 4 per cent.

Three days later a second mouse was killed. Weight 22·9 grm., length 83 mm., K content of the blood was at least 0·98 and not 1·07 per 1,000. A quite normal animal. Six fresh mice were added, making 10 in one cage; the strength of the solution was increased to 10 per cent.

After four days one mouse was killed: weight 20·16 grm., length 82 mm., and K content of the blood was at least 1·6 and not as much as 1·7 per 1,000. But much food was left uneaten each day. The strength of the solution was again increased to 20 per cent.

After two days one mouse was killed. Weight 15·05 grm., length 96 mm., very under-weighted, and the potassium content of the blood was at least 2·02 and not 2·1 per 1,000; the stomach and bowels were empty; the raised amount of K was probably due to starvation concentration.

On the next day a second mouse was killed. Weight 10·95 grm., length 78 mm., K content of the blood at least 2·3 and not as much as 2·6 grm. per 1,000; this animal was also under-weighted, the stomach and bowels were empty, and the raised potassium content of the blood was probably due to starvation concentration.

A third mouse showed a similar condition. Weight 9·86 grm., length 74 mm., and K content of the blood at least 2·4 and not 2·8 grm. per 1,000; the stomach and bowels were empty, and no trace of fat was noticeable on the body.

A fourth mouse was found dead and partly eaten.

A fifth mouse was killed. Weight 16·78 grm., length

86 mm., and the K content of the blood at least 1·6 and not 1·8 grm. per 1,000; the mouse was ill and under-weighted, but the stomach was full of food; it had probably eaten the dead mouse; it is noticeable that the K content is not so high as those mice which died with empty stomachs.

Four days later a sixth mouse was killed. Weight 17·62 grm., length 88 mm., and K content of the blood at least 1·8 and not 2·1 grm. per 1,000; this mouse was under-weighted, and had a raised amount of K in the blood. In this case I was able to obtain sufficient blood for further examination, and found Hb 118 per cent., red cells 15,300,000 per c.mm.; the animal had a starvation polycythæmia.

The next day two mice were found dead, both eaten, one picked to a skeleton. The surviving tenth mouse seemed well and was killed. Weight 22·38 grm., length 93 mm., K content of the blood at least 1·25 and not 1.42 grm. per 1,000; the Hb was 75 per cent.; apparently a normal quite unaffected animal, the K content of the blood being perhaps slightly raised for a mouse of this size.

The results of this series of experiments indicate that the mice objected to eat the bread soaked in potassium chloride, and were starved, but that by feeding on the dead mice the polycythæmia of the survivors could be removed, so that the well-fed mice showed no excess of K in their blood.

It is probable that the mice did not eat the bread soaked in the potassium chloride solution on account of its unpleasant taste, and it was suggested to me by Dr. Boycott that possibly by using a salt of potassium that is insoluble in water or alkali or saliva, but soluble in acids such as gastric juice, having no taste, it would more likely be eaten. Potassium metaphosphate, KPO_3, seemed suitable. One grm. of this salt contains 0·33 grm. of potassium.

Feeding with Potassium Metaphosphate. — Three sets of four mice were fed on water-soaked bread with which was mixed 1 grm. of KPO_3 in powder; so that if the mice ate equally and all the food was eaten, each mouse would receive roughly 0·08 grm. of potassium. On the following day one mouse in each cage was dead, and much food was uneaten. The autopsy in each case showed enteritis and peritonitis. The dose of the salt was probably too large.

Ten mice in one cage were fed on bread mixed with 0·5 grm. of KPO_3, so that if they ate equally and all the food was eaten, each mouse would receive roughly 0·006 grm. of potassium. On the following day all the food was eaten, and with one exception all the animals seemed well. Three days later (Monday morning) two mice were dead, one showing much post-mortem change, the other bore no evidence of enteritis or peritonitis. It was possible that the animal room had become too hot during the week-end interval. All the food was eaten, and the other remaining eight mice were well, and remained so till the tenth day of this diet, when one was killed. Weight 27·6 grm., length 100 mm., Hb 60 per cent., and K content of the blood at least 1·7 and not as much as 1·8 grm. per 1,000; the animal was well nourished, had probably no blood concentration, and the potassium in the blood was raised in amount, on the assumption that normally a mouse of this size would have only about 1·3 grm. K per 1,000.

The dose was therefore increased to 0·75 grm. of KPO_3 for the remaining seven mice, so that each mouse might receive about 0·1 of the salt, or about 0·03 grm. of potassium.

The animals remained well, and all the food was eaten. After the nineteenth day of this diet the seven mice were killed; the results of the blood examinations are given in Table III.

TABLE III.—RESULTS OBTAINED BY FEEDING MICE WITH METAPHOSPHATE OF POTASSIUM.

Mouse.	Fed for Number of Days.	Weight in grm.	Length in mm.	Hb. p.c.	"At least" amount of K per 1,000.	Mean amount of K per 1,000.	"Not as much" amount of K per 1,000.
1	10	27·6	100	60	1·66	1·73	1·80
2	19	28·25	92	65	2·40	2·49	2·58
3	19	28·35	95	75	1·67	1·72	1·76
4	19	12·86	77	80	2·10	2·35	2·60
5	20	17·60	85	70	2·60	2·75	2·90
6	20	22·72	90	72	1·98	2·05	2·13
7	20	19·82	83	76	1·80	1·89	1·98
8	20	14·65	76	80	1·91	2·10	2·29
Average		21·48	76	72	2·01	2·13	2·25

The table shows that the average weight of the eight mice was 21·48 grm., or nearly 4 grm. higher than that of the series of normal mice, and one might have expected a lower average potassium content of the blood; but it appears that the average mean potassium content of the blood of these eight mice fed with potassium metaphosphate is 2·13 grm. per 1,000, and the average mean potassium content of the blood of the 20 normal mice was 1·4 (or 1·36) grm. per 1,000, or 0·73–0·77 grm. per 1,000 less. But if of the 20 normal mice the eight heaviest are selected, their mean weights amounting to 20·14 grm., it is seen from the following table that their average mean potassium content of the blood is only 1·19 grm. per 1,000, or 0·94 grm., nearly 1 grm., per 1,000 less than the potassium-fed mice.

TABLE IV.

No. of Mouse.	Weight.	"At least" amount of K per 1,000.	Mean amount of K per 1,000.	"Not as much" K per 1,000.
	Grammes.			
20	24·75	1·26	1·32	1·39
11	21·49	1·06	1·10	1·14
19	20·71	1·16	1·29	1·42
13	20·00	1·02	1·10	1·19
12	19·24	0·84	0·91	0·98
17	18·48	1·02	1·06	1·11
4	18·70	1·19	1·34	1·50
8	17·79	1·20	1·40	1·60
Average	20·14	1·09	1·19	1·29
KPO_3 mice.	21·48	2·01	2·13	2·25

Therefore it would seem that the mice fed daily for about three weeks on bread to which is added 0·75 grm. of potassium metaphosphate, show at the end of that period an increase in the potassium content of their blood which on an average amounts to a mean amount of certainly 0·73–0·77 grm. per 1,000, and if the sizes of the mice are considered, this increase is about 0·94 or nearly 1 grm. of potassium per 1,000 (an increase of 80 per cent.). And this increase is probably in no way accounted for by a polycythæmia and starvation concentration of the blood, since the Hb per cent. in no case was raised, and the animals were

well nourished, and ate all their food during the period of the experiment.

It is therefore justifiable to assume that by feeding mice with potassium metaphosphate it is possible to raise the potassium content of their blood.

III.—THE INFLUENCE OF AN INCREASED POTASSIUM CONTENT OF THE BLOOD OF MICE ON THEIR READINESS TO "TAKE" AFTER TUMOUR INOCULATION.

Experiment 1.—Eight mice (Set A) received daily 0·5 grm. of KPO_3($\frac{0·5}{8}$ grm. per mouse), and after seven days the dose was increased to 0·75 grm.; one mouse died, but at the end of three weeks the remaining animals were well, and all the food had been eaten during the period.

These seven mice then received subcutaneously below the right axilla, by means of a needle canula, small portions of a tumour removed from another mouse; this tumour was firm and showed but little sign of necrosis; it had grown slowly and was about two months old.

Another seven mice (Set B) also received similar portions from this tumour in the same manner, and from this date these mice also were fed with 0·75 grm. of KPO_3 ($\frac{0·75}{7}$ grm. per mouse).

Another seven mice (Set C) also received portions of the same tumour in the same manner, but were fed on ordinary bread diet and received no KPO_3.

In these inoculations as far as possible each mouse received the same sized portion of tumour. The mice were not weighed, and no selection as to size was made, but no very small mice were used.

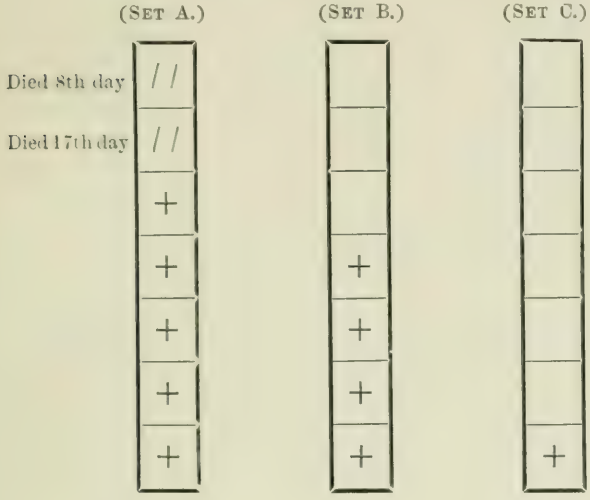
Eight days later no evidence of tumour growth could be detected in any of the mice; but after another six days, or fourteen days after inoculation, Set A showed tumours in five out of six mice (one mouse had died in the first week); the sixth mouse was ill and died two days later, and was found much eaten.

Set B showed tumours in four mice out of the seven.

In Set C no tumour could be detected in any of the seven mice; but on examination six days later one mouse of Set C had a tumour. No more tumours developed in any of the mice, up to a month after inoculation.

The results of this experiment may be conveniently expressed by the following diagram.

THREE WEEKS AFTER INOCULATION.

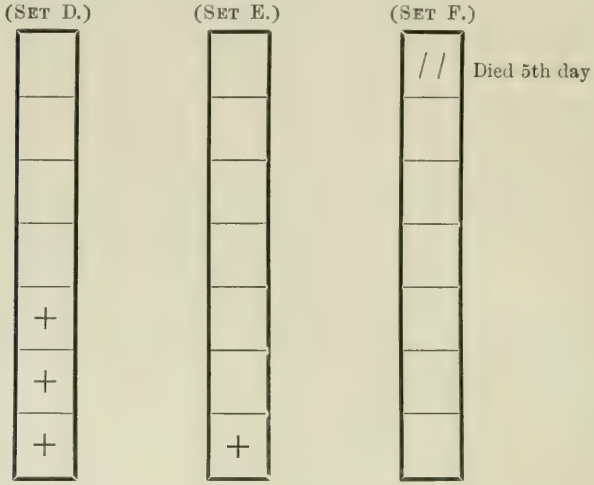


The experiment was now repeated. The tumour used for inoculation was of the same strain as that used in the previous series; it was six weeks old, of large size, and had been more rapid in its growth.

As before there were three sets of seven mice, D, E, and F: Set D had been fed daily for three weeks previous to the inoculation with 0·75 grm. of metaphosphate of potassium, Set E were fed with the same dose from the day of inoculation, and Set F received no KPO_3. Five days later one mouse in Set F was found dead; it had no sign of tumour growth. Examinations made a week after inoculation showed three mice with tumours in Set D, one mouse with tumour in Set E, but no mice in Set F showed any sign of tumour.

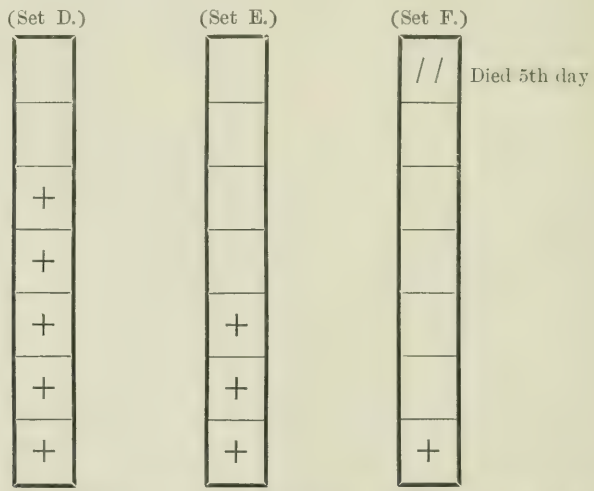
These results are expressed in the following diagram.

ONE WEEK AFTER INOCULATION.



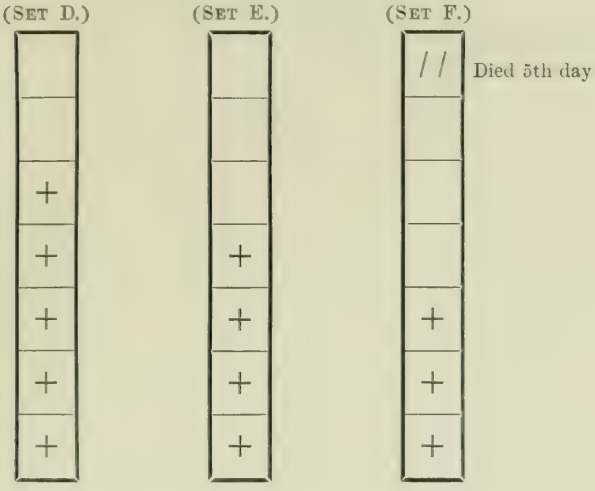
Three days later, ten days after inoculation, two more mice in Set D and two more mice in Set E showed tumours, and a small tumour could be felt in one of the mice in Set F.

TEN DAYS AFTER INOCULATION.



Four days later, fourteen days after inoculation, a fourth mouse in Set E showed a tumour. Seven days later, three weeks after inoculation, two more mice in Set F had tumours.

THREE WEEKS AFTER INOCULATION.



No more tumours developed in any of the mice.

This second experiment seems to corroborate the results obtained in the previous series.

The obvious criticism of these results is the uncertainty and possible variations in the amount of the tumour that was inoculated. In order to obviate this error, and also as far as possible to eliminate any personal bias, a third series was undertaken for me by Dr. Wedd. Three sets of mice, G, K, and M, were inoculated with 0·05 c.c. of an emulsion of tumour of the same strain as that used in the previous experiments.

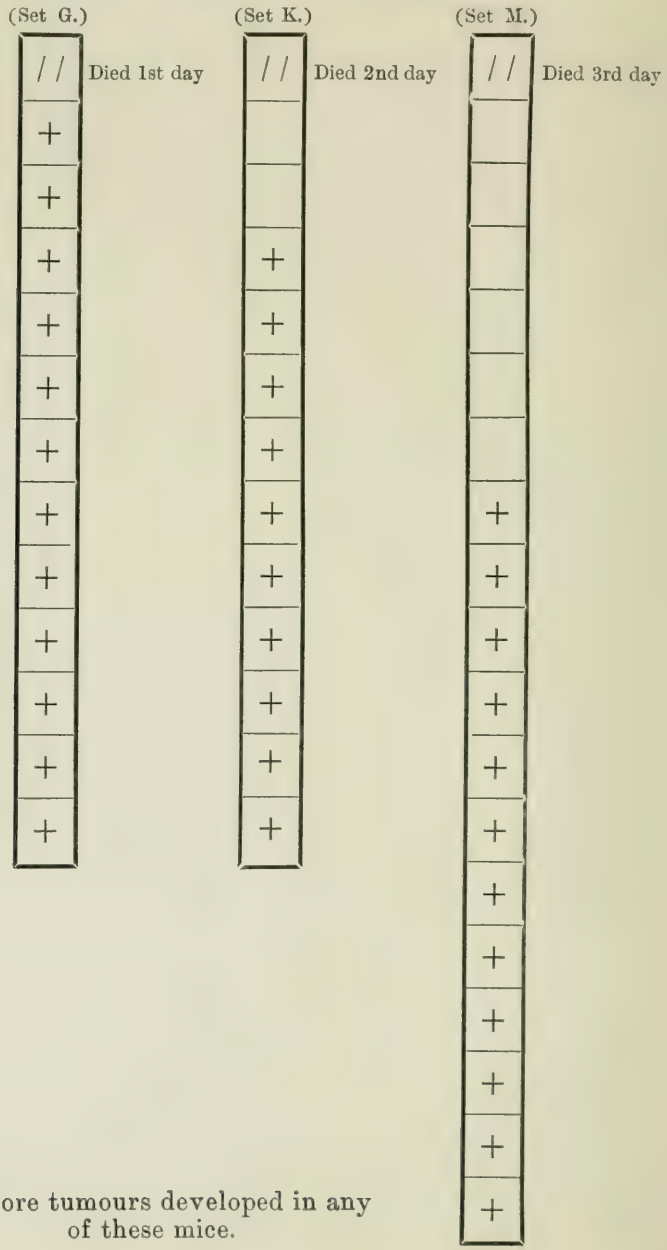
The 13 mice of Set G were fed daily for three weeks previous to and after the inoculation with 1·5 grm. ($\frac{1.5}{13}$ grm. per mouse) of metaphosphate of potassium; the 13 mice of Set K were fed from the day of inoculation with the same dose of KPO_3; and Set M, which included 19 mice, 6 of which were medium-sized, 7 larger, and 6 smaller mice, received no KPO_3.

This dose of tumour is large, and could be felt under the skin after injection. On the following day one mouse in Set G was dead; there was much post-mortem change, and the tumour-mass was soft and unattached. Two days later one mouse in Set K and one in Set M had died; in both cases the tumour-mass was soft and unattached.

Owing to the fact that the mass of inoculated tumour could be felt under the skin, examinations at early dates for the presence of definite tumours were not reliable; after

three weeks, however, the results were quite definite, and are shown in the accompanying diagram.

THREE WEEKS AFTER INOCULATION.



No more tumours developed in any of these mice.

Excluding those mice which died within the first three days after the inoculation, these results show that—

In Set G, 12 out of 12 mice had developed tumours, i.e. 100 per cent.

In Set K, 10 out of 12 mice had developed tumours, i.e. 83·3 per cent.

In Set M, 12 out of 18 mice had developed tumours, i.e. 66·6 per cent.

Grouping together the results obtained from these three experiments, and excluding those mice which died within the first three days, we find that among 26 mice fed on KPO_3 for three weeks previous to and after the inoculation with tumour, 22, or 84·5 per cent., developed tumours.

Among 26 mice fed on KPO_3 from the day of inoculation with tumour, 18, or 69·2 per cent., developed tumours; and among the 32 mice which received no KPO_3, 15, or 46·8 per cent., developed tumours.

Conclusions.

From these results I think it is reasonable to conclude that the administration of metaphosphate of potassium favours the development of tumours in mice which have been inoculated with portions of carcinomatous growth, both as regards the number of successful inoculations and as regards the date at which the tumours appear.

It was shown in the earlier experiments that feeding with metaphosphate of potassium increased the potassium content of the blood of mice, therefore an increase of the potassium content of the blood is favourable to the development of cancerous growth in mice.

AN UNUSUAL DISTRIBUTION OF SECONDARY INTESTINAL GROWTH IN A CASE OF SQUAMOUS-CELL CARCINOMA OF THE VULVA.

By HENRY BECKTON.

The case of squamous-cell carcinoma here described is interesting chiefly on account of the question which at once arises regarding mode of extension from the primary focus, the secondary growth in the intestines forming a long complete tube of almost uniform thickness lying between the peritoneal endothelium on the one hand and the muscular coats and mucous membrane on the other.

Clinical History.—The patient was a woman aged sixty-five years at the time of her death. After suffering for twelve years from excessive irritation in the vulva she noticed a small, acutely tender, and painful swelling of brownish colour in the right labium which tended to grow across the part; it was removed by operation a few days later, but soon recurred. No further operation was performed, however, until two and a half years after the first; the tumour was then again removed locally and the glands excised from the left groin. Recurrence was observed six months later in the left groin, and death took place a year after this, or four years after the first appearance of a tumour at the primary site.

Extracts from Post-mortem Notes.

External Appearances.—The body is emaciated. The anterior part of the vulva is the site of healthy scars due to old operations. The left lower limb is very œdematous, with a large excavated ulcer (diam. $1\frac{1}{2}$ in., depth $\frac{3}{4}$ in.), at the base of which lie the femoral artery and vein covered only by a thin layer of soft breaking-down tissue.

Thorax.—The *Heart* contains a small pale nodule (diam. $\frac{1}{8}$ in.) of (?) new growth in the myocardium and endocardium of the wall of the left ventricle.

Abdomen.—*Peritoneum*—The intestines are matted together, and the peritoneum generally is the seat of peritonitis of chronic character, the intestinal walls being everywhere much thickened and covered by numerous closely-set miliary nodules. The *Intestines* present thickened walls as just described, but the mucous membrane is normal. The *Liver* contains a few nodules of firm whitish new growth, some in the substance of the organ, some at the surface, the largest being about $\frac{3}{4}$ in. in diameter. The *Spleen* shows a small white mass at its surface (?) infarct, (?) new growth.

Pelvis.—*Peritoneum* shows general peritonitis as in the abdomen. *Uterus*—The cervix is ulcerated and hæmorrhagic in appearance. *Fallopian Tubes*—The left tube is thickened and studded externally with what appear to be nodules of new growth. The *Pelvic Wall* is extensively infiltrated on its left side with blood and (?) new growth.

Lymphatic System. — *Lumbar*, *Portal*, and *Bronchial Glands* are enlarged, apparently by new growth in the first two cases, doubtfully in the third.

Extracts from Histological Report.

Groin.—Section shows a small amount of new growth, but the tissue has for the most part undergone inflammatory and necrotic changes.

Pelvic Wall.—As preceding, except that new growth is here more conspicuous.

Lumbar Gland.—Lymphoid tissue is almost entirely replaced by new growth.

Portal Gland.—New growth present.

Bronchial Gland.—New growth present.

Liver.—Sections show secondary nodules.

Spleen.—Secondary growth present, a layer lying immediately beneath the peritoneal endothelium being specially noteworthy.

Diaphragm.—Section shows a peritoneal layer of new growth sending processes into the subjacent tissues, but uncovered by endothelium on the other side.

Peritoneal Adhesion.—Section shows a groundwork of connective tissue invaded by new growth.

Intestine (*Jejunum*).—The tissue lying between the peritoneal endothelium and the muscular coat is invaded, and for the most part replaced by new growth (*v. infra*). Similar appearances were found in ileum, vermiform appendix, and cæcum.

Heart.—Sections show new growth in heart-wall on inner aspect, originating apparently in the endocardium.

Bladder.—New growth is present in addition to inflammatory changes.

Uterus.—Section shows extensive invasion by new growth.

Fallopian Tube.—Section (longitudinal) shows new growth at its edges.

The histological characters of the new growth were those of squamous-cell carcinoma. It was of very cellular type; in fact, numerous sections were examined before any alveolar arrangement was discerned. Nevertheless, the neoplasm was of very slow growth, as evidenced by the time (four years) elapsing between the first operation and death; even then the nodules found in the liver were small.

The mode of spread was in the cases of most of the metastases apparently by the lymphatic vessels, along which the slow progress of the growth enabled it to reach the bronchial glands, though not the lungs; and the complete absence of secondary deposits from these seems to eliminate the blood stream as the possible source of the intestinal growth. The mechanism of the distribution of the cancer cells to form so uniform and extensive a layer of growth in the intestine, by the lymph stream or otherwise, is far from evident, and no fully satisfactory suggestion can be put forward to account for it; the appearances of this remarkable metastasis are therefore here simply placed on record.

AN EXPERIMENT ON THE TRANSPLANTATION OF MOUSE CARCINOMA.

By BRYDEN GLENDINING.

In a previous number of these Archives (vol. xix., Ninth Cancer Report), a mode of spread of abdominal carcinoma by the lumen of the Fallopian tube was recorded. Latterly an attempt has been made to reproduce a similar condition in cases of mouse cancer, and the following is a short account of the experiment.

A mouse tumour which from its life history in the laboratories was known to metastasise readily and which had the characters of a spheroidal cell adeno-carcinoma was chosen. From this tumour a piece was transplanted into the peritoneal cavity of eighteen mice at a point below the right costal margin in the mammary line, and the graft grew in the case of ten mice.

Before considering the results of such implantations and their secondary growths, the different relationship of the mouth of the Fallopian tube to the ovary and the position of the appendages in the abdominal cavity in the human and mouse species should be noted.

Whereas in the human species the abdominal ostium of the Fallopian tube opens freely in the peritoneal cavity, in the mouse, on the other hand, the ostium of the Fallopian tube opens into a pouch, which again opens into the peritoneal cavity.

Again, whereas in the human species the Fallopian tube comes to occupy a position near the lowest part of the peritoneal cavity, where the effect of gravity plays a considerable part in carrying down any free bodies in that cavity, in the plantigrade group of mammalia, on the other hand, the Fallopian tube orifices are found in the lumbar region occupying a position which, in these animals, is near the summit of the abdominal cavity.

The following table shows the position of the new growth found post-mortem in the experimental mice :—

Abdominal metastases—	
Mesentery of Small Intestine	6
,, Colon	7
,, Spleen	3
,, Stomach	3
Liver (anterior surface)	1
Kidney	2
Anterior Abdominal Wall	1
Central Tendon of Diaphragm	1
Mesentery of Uterine Horn and Tube	3
,, Fallopian Tube	3
Ovary	3
Bladder	2

It will be seen that metastases occurred in relation to the Fallopian tube and ovary in only three cases, and in each case the new growth was represented by a mass occupying principally the mesentery of the Fallopian tube and ovary and only involving them by direct extension.

In no case was the growth found free in the lumen of the Fallopian tube, nor were any isolated nodules seen which suggested a permeation by means of the lymphatics.

Free fluid was never encountered in the abdominal cavity. In only one case did metastases occur outside the abdominal cavity, when they were found upon the pleura and pericardium.

The new growth in the ovary produced small masses occupying the stroma and having the characters of metastases elsewhere. The ovary did not show great enlargement or any cystic formation.

Experiments to reproduce the human condition in mice were therefore unsuccessful.

ON PRE-OPERATIVE VACCINATION IN CARCINOMA CERVICIS.

By BRYDEN GLENDINING.

In the extensive operation known as Wertheim's Hysterectomy, which has been largely performed during the last few years both at the Middlesex Hospital and the Chelsea Hospital for Women, it was noted that the proportion of cases in which inflammatory products formed either in the abdominal wound or in the pelvic operation area was high, and this in spite of the most rigid aseptic precautions in operative technique. Further it was observed that, in certain cases when cultivations were made, the organism obtained from the suppurative foci was identical with that present, secondarily, in the new growth. It was consequently surmised that, owing to the severe nature of the operation, the patient's resistance was much lowered to the infecting agent, and that any measures which served to heighten this resistance would be of great advantage.

The measure suggested by the operators was the preparation of vaccines from the infecting agents and their injection anteriorly to operative interference.

The results of such pre-operative vaccination are embodied in the present paper, and comparison is drawn from cases operated on concurrently, which were not vaccinated.

The patient, immediately upon admission, has a culture taken from the cervical growth, and from the organism present a vaccine is prepared. This vaccine is injected on a single occasion at least five days, but in several cases six or seven days, before operation. The dose of the vaccine has varied both according to the nature of the infecting organism and also for the same organism. Starting with a medium dose of thirty million streptococci, or fifty million staphylococci, a change was made for some time, reducing the dose to fifteen million streptococci or thirty million staphylococci;

but as the effect in one or two instances did not seem so good, a return has been made to large doses, and latterly the dosage thought to be most suitable for single injections has been fifty million streptococci and ninety million staphylococci.

The vaccine has in every case been an autogenous preparation. The nature of the organism cultivated in the 20 cases was—

Streptococcus	10
Staphylococcus albus	7
Staphylococcus aureus	2
Streptococcus and Staph. albus ...	1

An analysis of the cases so treated, compared with the untreated cases operated upon during the same period, appears distinctly to favour the vaccine therapy.

Eliminating all cases of death in both classes from shock or accidental causes, e.g. embolism, there were no deaths in the present series of 20 vaccinated cases, whereas there were 7 deaths in the 30 untreated cases. Of these 7 cases, 5 showed pyrexia, but it is not quite clear that the condition which caused it was either streptococcal or staphylococcal. In the 2 remaining cases this objection does not hold, and therefore it may safely be concluded that the mortality in the untreated cases was greater than in the vaccinated cases, though it is uncertain by how much.

Inflammatory lesions occurred in only 3 cases (15 per cent.) of the vaccinated class. In the most marked case a slough formed in the upper part of the abdominal wound and an offensive vaginal discharge, indicative of suppuration in the pelvic operation area, was also present. The vaccine had been a single injection of fifty million (*Staphylococcus albus*). In the second case an acute vulvo-vaginitis occurred, but as no culture was made the nature of the infecting organism was not determined. In the third case, six months after operation, the patient returned with a mass the size of an orange in the abdominal scar. It was thought to be malignant, and was removed, but proved on histological examination to be an inflammatory mass. All these cases recovered.

Comparing this with the cases of inflammatory lesions such as suppuration in the abdominal wound or in the pelvic tissues, it is found that the number is higher, being actually

nine cases (30 per cent.), representing double the frequency of such lesions in treated cases.

Regarding the temperature chart during the entire stay of the patient in hospital as an index of progress in convalescence, it is found that complete absence of pyrexia was observed in 40 per cent. of the vaccinated cases, whereas in the non-treated cases this condition was seen in only 13·3 per cent. of the cases. Again, as the majority of inflammatory lesions which arise do so in the first week after operation, during which period the vaccine may also be assumed to be

CASES OF WERTHEIM'S HYSTERECTOMY TREATED WITH AUTOGENOUS VACCINES BEFORE OPERATION.

No.	Organism and Dose of Vaccine given, in millions.	Temperature.	Result. C. = Convalescent. D. = Died.	Remarks.
1	Streptococcus, 50	Normal 21 days, then slight pyrexia.	C.	Vesico-vaginal fistula
2	Staph. albus, 30	4–12 days, 102° continuous	C.	Glands malignant.
3	Streptococcus, 30	7–28 days, remittent ...	C.	Inflammatory mass removed from abdominal scar six months later.
4	Streptococcus, 50	1–25 days, 102° continuous	C.	Acute vulvo-vaginitis.
5	Streptococcus, 30	1–20 days, 100° remittent	C.	—
6	Staph. albus, 50	1–12 days, highest 101° ...	C.	Slough in upper part abdominal wound, offensive vaginal discharge.
7	Streptococcus, 40	8–33 days, highest 102° ...	C.	—
8	Streptococcus, 15 / Bacillus coli, 40	None	C.	Blood in urine a few days.
9	Streptococcus, 16	19–21 days, highest 101°	C.	Retention urine.
10	Streptococcus, 25	No pyrexia	C.	Retention urine.
11	Staph. albus, 25	7–17 days above 103° ...	C.	—
12	Staph. aureus, 70	No pyrexia	D.	Heart failure second day.
13	Staph. albus, 100	No pyrexia	C.	—
14	Streptococcus, 40 / Staph. albus, 100	1–21 days, pyrexia with rigors.	C.	Bronchitis.
15	Staph. albus, 90	1–4 days, 100°	C.	—
16	Staph. albus, 100	No pyrexia	C.	—
17	Streptococcus, 50	20–30 days, pyrexia ...	C.	Cystitis 20th day, cleared up with Bacillus coli vaccine
18	Staph. albus, 100	No pyrexia	D.	Heart failure seventh day.
19	Staph. albus, 150	No pyrexia	C.	—
20	Staph. aureus, 500	No pyrexia	C.	

most effective, the temperature chart of the first week after operation may be used for comparison. In the vaccinated series there was absence of rise of temperature during the first week in 70 per cent. of cases, while in the non-vaccinated cases absence of rise of temperature was noted in 23·3 per cent. only.

In one case a vaccine consisting of streptococci and *B. coli communis*, both obtained from the culture made from the cervix, was given with good result in convalesence. In another case in which pyrexia occurred on the 20th day, a specially prepared vaccine of *B. coli communis* was administered with highly beneficial result.

In conclusion it may be stated that the results of pre-operative vaccination in the present series appear good, but that a still more perfect convalescence may possibly be obtained by a pre-operative injection of a mixed vaccine derived from the infecting organism of the cervical growth on the one hand and from the *B. coli communis* on the other.

A NOTE ON CARCINOMA OF THE UTERUS AND CERVIX.

By BRYDEN GLENDINING.

This paper constitutes an inquiry into the relative frequency of columnar-cell carcinoma of the cervix, and embodies a criticism that the percentage of cases usually given is abnormally high.

In the first instance steps were taken to try to define the limits of the columnar epithelium of the cervix, both below, where it passes into the squamous covering of the cervix, and especially above, where it merges with that of the body of the uterus. For this purpose material obtained from the post-mortem room, the operating theatre, and from infants still-born, has been extensively employed.

Determination of the line of demarcation between the squamous covering of the lower cervix and the columnar epithelium of the endocervix presented little difficulty. The lowest level at which the columnar epithelium was found was always distinctly within the external os, and the highest about an inch up from the external orifice. Between these two levels great variation was found even in the same cervix; invariably the extent of the squamous epithelium was different on the two sides as well as anteriorly and posteriorly. In respect of the upper limits of the cervical columnar epithelium, it at once became evident that ordinary histological stains could not be relied upon as a means of distinguishing the body epithelium from that of the cervix. Consequently a histo-chemical method was adopted by which chemical characters of the epithelium in the respective regions were thrown into relief. For this purpose Mayer's muci-carmine solution was used, which depends upon the affinity of mucin

for carmine. The mucous cytoplasm stains a bright pink, especially in its free border. This was found reliable, and showed readily the distinction between the mucous epithelium of the cervix and the columnar epithelium of the body. Here again the line of demarcation varied upwards and downwards as much as an inch in different segments of the same cervix.]

While the division of carcinomata of the cervix into two broad groups, as they arise respectively in the columnar or squamous-covered regions, is readily made, the distinction between the columnar-cell growth of the cervix and that of the body presents much more difficulty. This difficulty owes its existence to two facts:—(1) The morphological similarity of the epithelium from which the two growths arise; (2) The obscuring of certain chemical characters in the cells incident upon abeyance of functional activity in the new growths. Hence the mucus-secretion, which with suitable staining serves to distinguish normal cervical epithelium, fails to some degree when that epithelium becomes carcinomatous.

The following details are drawn from 168 cases of uterine and cervical cancer which have been examined during the last three years. The figures represent early cases, since only those in which a complete hysterectomy was done have been included. The advantage of considering early cases is that in them the site at which the growth originated has as yet in the majority of cases become little obscured by extension into neighbouring regions.

The method of fixation found most suitable for application of the muci-carmine stain was that obtained by increasing strengths of methylated spirit. Fixation of the tissues in formalin did not give such good results, although this method does not altogether obscure the reaction in the case of the normal cervical glands. Other methods of fixation, such as acetic alcohol, gave indifferent results.

In considering the histological appearances, it is at once evident that the success or failure of the reaction when applied to new growths depends upon the degree to which the original histological characters are reproduced. In this respect it must be admitted that in rapidly growing areas with rich cellularity and little differentiation of cells the

stain is a failure; there is here no mucin to stain. Again, in portions of the growth where degenerative changes are marked, the staining effect is identical whether the growth have come from the cervix or from the body. However, between these two extremes, one or more areas, variable in extent, are to be found in each cervical growth at which the specific staining reaction is recognisable.

The typical appearances in a section of the cervix the seat of columnar-cell carcinoma, stained by muci-carmine, are as follows:—In the more specialised areas of gland formation, the epithelium bordering the spaces is seen with a spherical nucleus situated near the middle of the cell and surrounded by a bright pink protoplasm of variable extent. This bright pink staining stands out in contrast with the coloration of the débris present in the lumen of the gland, and with the cytoplasmic staining of adjacent heaped masses of carcinoma cells. If the section has been taken at the growing edge, a normal cervical gland may often be seen, and the staining effects compared. The difference is one of form and not of colour, the normal cell having the nucleus near the base of the lining epithelium, and thus leaving a large area of typical bright pink protoplasm to form the boundary to the gland space. The material present in the gland varies in its affinity for the stain, sometimes taking on a bright pink hue; but more commonly it is a pale pink, similar to the connective tissue structures.

In two of the cases of columnar carcinoma of the cervix metastases were present in the iliac glands. In one of these, although the original gave the staining reaction, the secondary growth simply stained a uniform pale pink colour. In the second case, the metastatic deposit was small in amount, and showed a well-formed gland lumen; the cells at one side of the lumen were of a fairly characteristic pink colour.

The typical appearance of columnar-cell carcinoma of the body stained by the muci-carmine method is a uniform pale pink coloration of the cytoplasm and of the secretion and débris present in the gland spaces. There is no sharp contrast between the carcinomatous epithelium lining the gland spaces and the rest of the growth as in the case of the cervix.

Examined by this method 168 cases of operable carcinoma afforded the following information :—

		Cases.	p.c.
Carcinoma of body.	Columnar - - -	27	16·6
	Malignant adeno-myoma	1	
Carcinoma of cervix.	Columnar - - -	6	3·6
	Squamous - - -	134	79·8
		168	

These figures are lower for carcinoma of columnar origin in both situations than those given by Leitch and Andriesen (Archiv. Middlesex Hosp., Fifth Cancer Report, p. 165), and by MacCormac (Archiv. Middlesex Hosp., Eighth Cancer Report, p. 20). These authors, however, did not use the muci-carmine method.

VARIATIONS IN THE COAGULATION OF THE BLOOD IN NORMAL INDIVIDUALS AS ESTIMATED BY THE METHOD OF DALE AND LAIDLAW.

By ELIZABETH H. LEPPER.

In order to determine slight variations in the coagulability of the blood a method is necessary in which the experimental error is small, and at the same time in which the technique is not unduly difficult. After a preliminary examination of several suggested methods such desiderata appeared to be supplied by the method of Dale and Laidlaw,* the principle of which is as follows: A capillary tube of a certain length and bore, partially closed at each end so as to imprison a small pellet of shot, is filled with blood, the ends of the tube are completely closed by a pair of forceps, and the capillary submerged in a bath of warm water. By gently rocking the tube the shot is made to run up and down until, a certain amount of fibrin having formed, it ceases to move, and indicates the onset of coagulation.

This method was therefore carefully considered, and it was found that in order to get the least possible error certain precautions had to be taken. Subject to these the method appears to be reliable to within about 5 per cent.

(1) **The Shot.**—Shot varying from 9 mgr. to 9·6 mgr. in weight were used; those which appeared to be about the right size were picked out and tested in a glass gauge which had been graduated with shot of known weight. It was found that trusting to the eye alone did not give sufficiently

* "A Simple Coagulometer," H. H. Dale and P. R. Laidlaw, "Journal of Pathology and Bacteriology," vol. xvi., No. 3.

accurate results. The shot were washed in water, methylated spirit, absolute alcohol, and ether, dried, and kept in a clean glass box till wanted.

(2) **The Capillary Tubing.**—As regards the glass tubing, only those pieces were selected which had a diameter of 1·35 mm. at each end, or 1·3 mm. at one end and 1·35 mm. at the other. These tubes containing the shot described were found to give the most even results, though it is quite possible to use tubing 1·3 mm. or 1·4 mm. in diameter by selecting smaller or larger shot to match. The size originally chosen was determined by the capillary tubing which was available. The tubes, when complete, were about 1·8 cm. in length.

(3) **Forceps,** for holding the capillary in the water-bath after it had been filled with blood, were made from a pair of Cornet forceps by straightening out the blades and decreasing the strength of the spring by flattening the handle so that the points remained a third of an inch apart. A piece of narrow rubber tubing half an inch long was then slipped over the end of each blade, so that when the filled tube was caught between the forceps a rubber surface was pressed firmly over each opening and prevented the entrance of any water.

(4) **Method of obtaining the Blood.**—A triangular surgical needle was used. It was washed after each prick in water, and kept in absolute alcohol. It was found to be of the greatest importance that the needle should be clean and also sharp. Upon this point I agree with Dale and Laidlaw that a blunt needle introduces uncertainty of results and acceleration of clotting.

The palmar surface of the end of a finger was washed with ether and absolute alcohol and allowed to dry. A bandage was applied sufficiently tightly to produce a feeling of fullness in the finger-tip.

As soon as the finger had been pricked the stop-watch was started and the blood as it flowed out collected in the tube. When this had been completely filled the forceps were applied, and 15 sec. after starting the watch the tube was put in the bath. It is often possible to prick the finger, fill the tube in 4 sec., and have it in the bath in 8 sec.; but at the bedside

conditions are not so favourable, and it was found that 15 sec. gave only just enough time for the various steps. The length of time which elapses between the prick and the blood reaching the hot water makes a difference in the coagulation rate, as the following figures show in the two cases A and B :—

	Length of time before Tube reached Bath.	Time taken to Coagulate.
A	30 sec.	112 sec.
	30 ,,	111·8 ,,
	15 ,,	100·2 ,,
	15 ,,	100·2 ,,
B	25 ,,	114·2 ,,
	25 ,,	111·8 ,,
	15 ,,	101 ,,
	15 ,,	100 ,,

Such results were found when the blood was easily obtained and the delay occurred in the application of the forceps. When, however, a large enough drop was not available at once and pressure had to be made, the time, instead of being prolonged, was often shorter than usual. In order therefore to get comparable results in different people it was necessary to fix a time-period at the end of which the tube should be put in the bath, and as 15 sec. was found to give enough time in all cases this period was chosen.

The importance of taking the first drop.—A fresh prick had to be made for each observation, as the second or third drop always coagulated more rapidly than the first, even if the finger had been well washed with water, alcohol, and ether in between. This is shown below :—

Time taken to Clot by 1st drop.	Delay.	Time taken by 2nd drop.	Delay.	Time taken by 3rd drop.
101·2 sec.	5 min.	90 sec.	2 min.	63·6 sec.
104·4 ,,	4 ,,	92·6 ,,	11 ,,	84·8 ,,

In each instance the finger was washed with water, ether, and alcohol, between each drop.

The degree of congestion of the finger.—It was found that if the bandage were applied sufficiently tightly to render the

blood dark in colour, the coagulation rate was shorter than usual. The following observations illustrate this point:—

Coagulation Rate when Blood was Red.	Period of Congestion.	Rate when Blood was Dark.
(1) 103·4 sec.	21 min.	97·6 sec.
(2) 102·6 ,,	9 ,,	96·6 ,,
(3) 101 ,,	1·5 ,,	97·8 ,,
(4) 103·6 ,,	1·7 ,,	99·8 ,,
(5) 104·6 ,,	1·5 ,,	93·4 ,,
(6) 105·6 ,,	1·7 ,,	97·2 ,,

Provided the blood looked dark there was always a slight decrease in the rate of clotting, from 3 to 11 per cent. The length of time the finger was congested did not appear to have much effect.

It is important therefore when obtaining the blood to produce hyperæmia without obvious darkening of the blood.

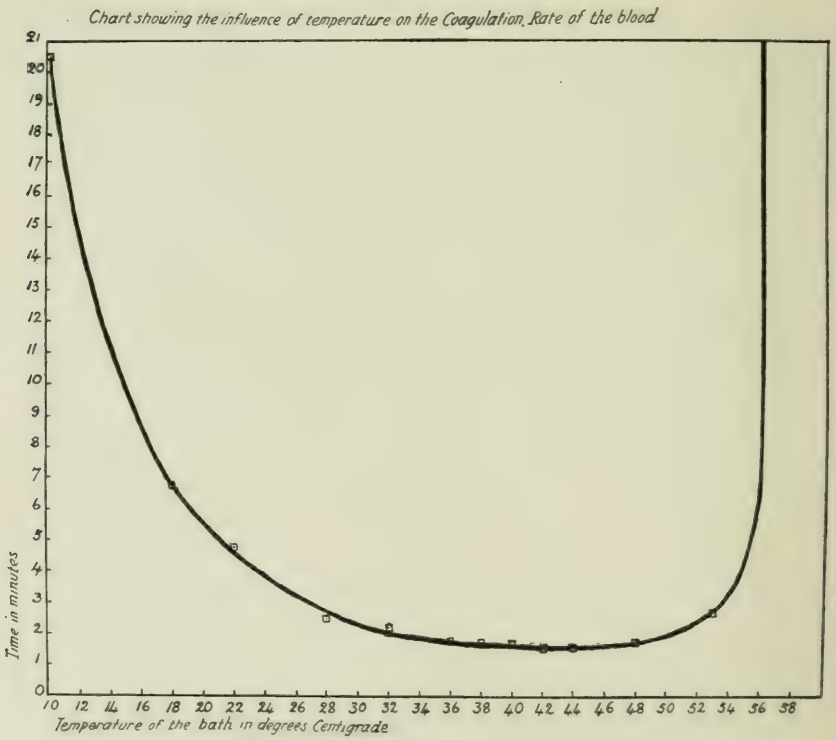


FIG. 1.

(5) **The temperature at which the observations were made.** The coagulation rate of the blood varies greatly at different temperatures, even one degree rise or fall making a considerable alteration in the time taken. It was necessary therefore to select a temperature at which slight variations in the bath had the least effect. In the accompanying chart (Fig. 1) the influence of temperature on the coagulation rate of the blood is shown. It will be seen that the curve is flattest between 36° C. and 48° C. At 36° C. the rate is 110 sec., at 40° C. 102 sec., a difference of 8 sec. for 4° alteration in temperature, or rather less than 2 per cent. variation in the rate for 1° change in the bath; that is to say, a possible error of 2 per cent., should the water vary 1° C. during the period in which experiments are being made. (Fig. 1.)

At 18° C. the rate is 409 sec., at 22° C. 289 sec., a difference of 120 sec., or 30 sec. per 1° C. change, which on the mean time of 349 sec. is about 8·5 per cent. variation for each degree. The possible error therefore due to slight changes in the temperature of the bath is four times as great at the lower temperature.

Chart to show the variations at the higher temperatures on a large scale.

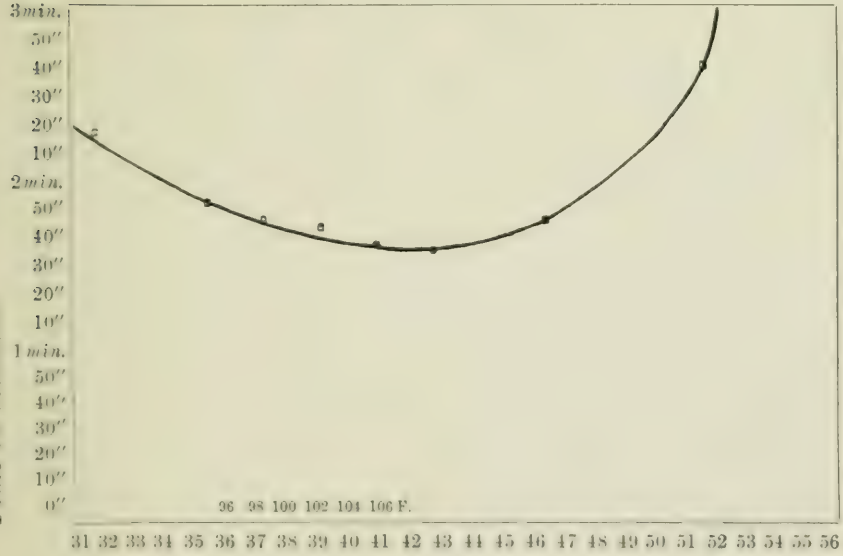


FIG. 2.

The temperature selected for the bath was therefore 37·5° to 38·5° C. The further advantages of using a bath at this temperature are that the end point is generally very abrupt, and that several observations can be made without fatiguing the patient.

A portion of the temperature curve is repeated on a large scale (Fig. 2) to show the effect on the coagulation rate of temperatures such as occur during disease, 96° F. to 104° F. The changes will be seen to be very slight. (Fig. 2.)

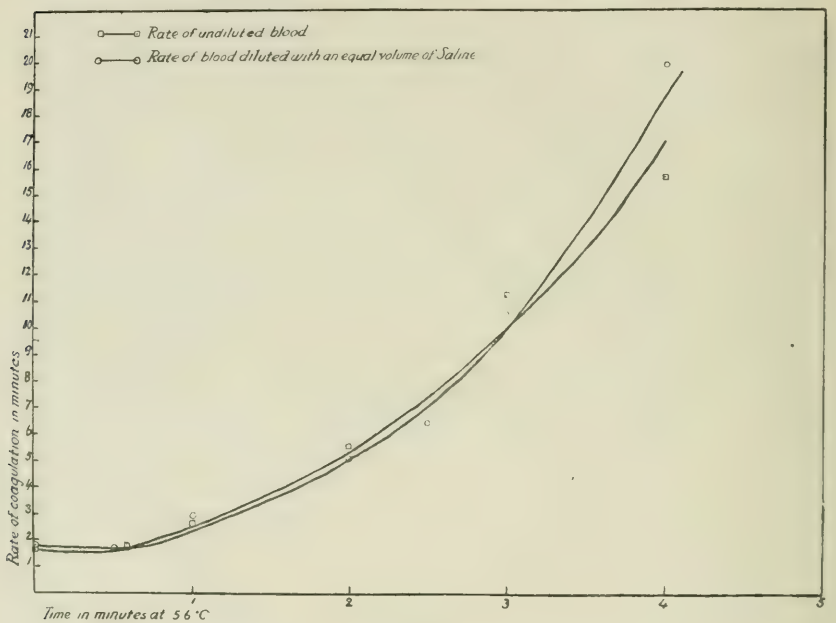


FIG. 3.

It will be noticed that above 45° C. the coagulation rate becomes slower and slower, until at 55° C. no clotting at all takes place. One of the tubes which had been kept at this temperature for one and a half hours without any sign of coagulation was removed and kept at room temperature for 48 hours, at the end of which time the blood was still quite fluid.

This non-coagulability of the blood at first was thought to be due to the destruction of prothrombin which Mellanby * found takes place at 55° C.; but, whatever the nature of the

* "The Coagulation of the Blood," J. Mellanby, "Journal of Physiology," Dec. 1908.

change, it is not an instantaneous one, for an immersion for two or three minutes in a bath at 55° C. is not sufficient to deprive the blood of its coagulability, although the rate of clotting is delayed.

A series of experiments was carried out in which the delay in the onset of coagulation, produced by submersion in a bath at 56° C. for varying periods of time, was estimated, the experiments being completed at 38° C. The results are plotted in Fig. 3.

It will be seen that keeping the blood at 56° C. for 30 sec. has, if anything, an accelerating effect on the coagulation rate, suggesting an increase in the rate at which fibrin ferment is produced, but that longer periods cause increasing delay in the onset of clotting, pointing to the rapid destruction of the ferment once it has been produced.

It would, however, be necessary to isolate the several substances concerned in the process of coagulation in order to determine the exact influence of the temperature.

The experiments were repeated with blood which had been diluted with an equal quantity of saline, and similar results were obtained.

The Coagulation Rate of the Blood in Health.

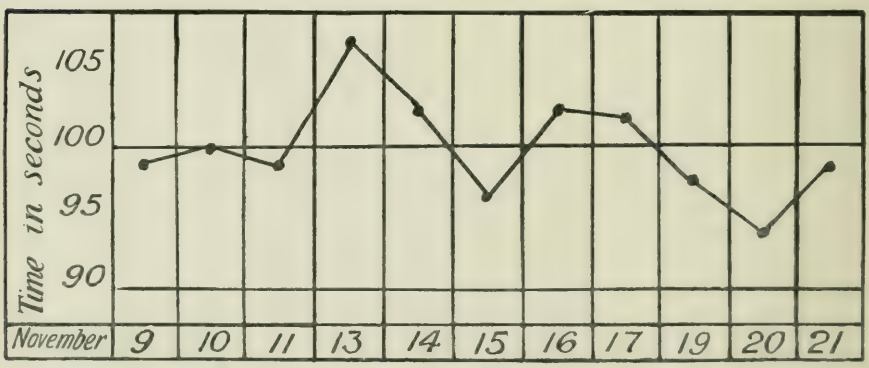
The importance of all these details as regards the size of the tubes, obtaining the blood, and the temperature at which the observations were to be made having been determined, estimations of the blood-coagulation rate were made on normal persons. Fifty healthy people were examined, two observations being made in each instance, and the mean taken. The average rate of coagulation was 99·2 sec., the longest time was 109·6 sec., the shortest 86·8 sec., the difference being 22·8 sec., or 23 per cent. on the mean time; 44 of the rates obtained varied between 92 sec. and 107 sec., that is to say 88 per cent. showed a variation of 15 sec. only. The average error on the two observations made was 2·7 per cent., the highest being 9·3 per cent., the lowest 0 per cent. This does not include some twelve cases which when first examined gave a large error. These persons were re-examined at a later date, and more even results were

obtained. The average error including these cases was 4·2 per cent.

As a contrast to these normal people, a boy who was known to be hæmophilic was examined on several different days; his rate varied between $8\frac{1}{4}$ and $9\frac{3}{4}$ min. A second boy, who suffered from severe attacks of purpura, showed a delay of only 20 per cent. on the normal time, coagulation taking place in 120 sec.

(1) **The influence of age.**—The average age of the 50 individuals was 24·6 years; in eight cases, age 30 to 45 (the average age being 32·6 years), the mean rate was 99·3 as against 99·2 sec. on the total number; in ten persons, age 45 to 64 (the average age being 53·6 years), the mean rate was 101·2 sec., the longest 108·5 sec., the shortest 92·6 sec., the difference being 15·9 sec., or 15·7 per cent. on the mean time. The average error in these cases was 3·3 per cent., the highest being 7·8 per cent., the smallest 0·4 per cent.

(2) **Daily variations.**—A series of daily observations, extending over three months, and numbering 107 experiments, were made in one individual to test the reliability of the method, and to see whether physiological variations could be detected. For each observation the mean of the rates given by two different tubes was taken. The average time was 100·3 sec., the longest 108 sec., the shortest 93·5 sec., a difference of 14·5 sec., i.e. a possible variation of 14·5 per cent. in a normal



Daily chart of L. L. showing greatest variation in the series of 107 consecutive observations.

FIG. 4.

person. On the other hand, 87 per cent. of the rates obtained were between 97·5 sec. and 105 sec., a variation of 7·5 per cent. only. The most irregular part of the chart is reproduced (Fig. 4).

(3) **The influence of the time of day.**—Observations were made at various periods of the day, between 7.30 a.m. and 12 midnight, but no constant alteration could be detected, and the mean of several morning and evening observations showed no change.

Date.	Morning rate.	Evening rate.
22nd Sept., 1911 ...	103 sec. ...	102·7 sec.
26th Sept., 1911 ...	100·3 ,, ...	104·7 ,,
27th Sept., 1911 ...	101·6 ,, ...	101 ,,
28th Sept., 1911 ...	100·3 ,, ...	104·1 ,,
30th Sept., 1911 ...	100·2 ,, ...	100·2 ,,
2nd Oct., 1911 ...	100·2 ,, ...	99·2 ,,
3rd Oct., 1911 ...	101·5 ,, ...	98 ,,

Mean of 7 morning observations, 101 sec.
,, evening ,, 101·4 ,,

(4) **The influence of the external temperature.**—During the series of daily observations the room temperature varied from 28° C. to 9° C., but no alteration in the coagulation rate could be correlated with these changes.

(5) **Food and drink** do not appear to have any effect. Numerous experiments were carried out before and after meals, but all the results fell within the normal limits of variation.

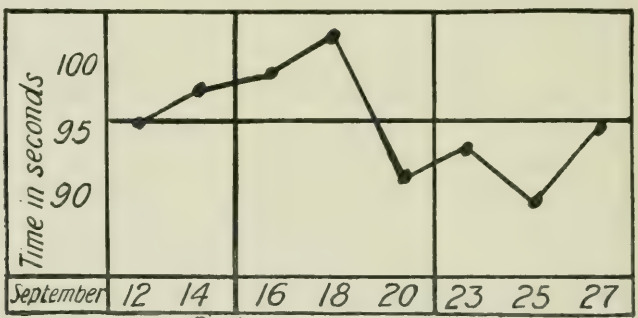
(6) As the patients examined were women, it was thought advisable to investigate the **coagulation rate of the blood during menstruation.** Estimations were made in ten different women every other day in the week preceding the period, every day for the first three days, and then every other day till a week after the end of menstruation.

The mean of the observations made during the period when contrasted with the mean of the whole series of experiments did not show any marked alteration; in two cases it was slightly longer, in three slightly shorter, in four no change. In the tenth case, which was examined over four

consecutive menstrual periods, it was twice longer and twice no change.

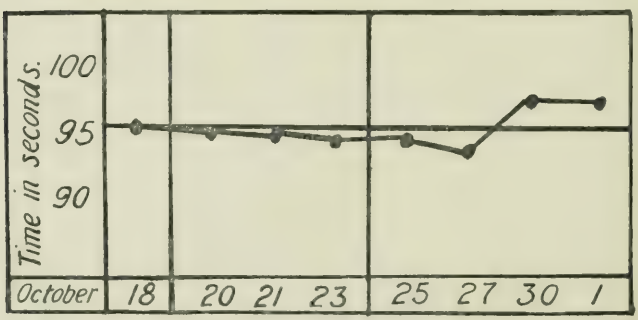
Although the mean times do not indicate any variation, yet the charts of these cases show that in seven of the ten cases the longest coagulation rate observed in those individuals occurred during the period. In four cases the rate of clotting was slowest the day before or the first day of the period, and in three cases there was a gradual lengthening until the maximum was reached on the fourth day of the period. A much larger number of cases would have to be investigated before any definite conclusion could be arrived at, but there is some evidence that the coagulation rate is generally rather longer during the period or just before it.

These cases were also of value in showing the daily variation one may expect to find in normal people. One case has



Daily chart of D. showing greatest variation, 12.8%

FIG. 5.



Daily chart of V. I., showing least variation, 4.1%

FIG. 6.

already been fully described. The other nine cases were each examined on from 8 to 14 different days, average observations on ten different days in each. The greatest variation shown was 12·8 per cent., the least 4·1 per cent. The charts of these two cases are reproduced (Fig. 5 and 6). The quickest rate found in any case was 90·4 sec.

In only 13 observations out of 96 experiments was the rate shorter than 95 sec., i.e. 14·4 per cent., and consequently if a case be examined on three different occasions the probability that a sequence of three short coagulation rates is a mere coincidence is slight.

On the other hand, if only one observation be made, the rate obtained may be 5 per cent. longer or shorter than the mean rate for that person, for the average variation on the nine cases was 9·4 between the extremes.

Effect of Drugs.

(7) **The influence of certain chemical substances.**—*Calcium Salts.*—Calcium chloride was administered to two people, A and B, whose coagulation rates had been charted for some time, so that their individual variation was known. In all the following experiments on B, the observer did not know what substances, if any, had been employed until the end of the experiments. A had an average coagulation rate of 100 sec., the greatest variation being 6½ sec. below and 8 sec. above, about 90 per cent. of the observations, however, not exceeding 4 per cent. above or below the mean. Nine grammes of calcium chloride were taken in three days; the variation was from 1 per cent. below to 2 per cent. above the mean. Eight grammes of calcium lactate were given in four days; the variation was from 4 per cent. below to 7·5 per cent. above the mean.

B had an average coagulation rate of 97 sec.; the greatest variation found was 7 sec. above or below the mean, 80 per cent. of the observations falling within 5 per cent. above or below the mean. Five and a half grammes of calcium chloride were taken in seven days; the variation was from 3 sec. below to 2 sec. above the mean.

Quinine.—As quinine has an inhibitory effect on the movements of the leucocytes, it was thought possible that it

might have some influence on the rate of clotting. A was given 1½ grammes in thirty-six hours, and developed noises in the head with slight deafness; the variation was from 4 sec. below to 2·5 sec. above. B had 2·2 grammes within two days on two separate occasions; the variation was from 5 sec. below to 7 sec. above the mean.

Aspirin.—A had 1½ grammes in forty-eight hours; the variation was from 2 sec. below to 4 sec. above the mean. B had 4 grammes in three days; the variation was from 2 sec. below to the mean.

Citric Acid.—A was given this substance on three different occasions. After the first administration the coagulation rate remained from 3 sec. to 8 sec. above the mean, but on subsequent occasions it varied from the mean to 6 sec. above it. B had 14 grammes of sodium citrate in eight days; the variation was from 5 sec. below to 2 sec. above the mean.

In none of the above experiments could any alterations be detected which were not explicable by normal variations in the coagulation rate.

(8) **The effect of the addition of certain substances in vitro.** —(a) *Normal Saline.*—The negative results obtained by the administration of calcium chloride by the mouth made it of interest to try the effect of the addition of solution of that substance directly to the blood.

Some preliminary experiments were carried out to find what effect on the coagulation rate would be produced by the addition of an equal volume of normal saline solution to the blood. The method adopted was to measure the length of the capillary tube, half fill it with saline, prick the finger, and run in blood till the tube was completely full, and test the coagulation rate in the usual way. The excursion of the shot caused the blood and saline to be thoroughly mixed.

It was found that the dilution of the blood only caused a delay of from 5 to 10 per cent. in the coagulation rate, the curves of coagulability at different temperatures being parallel (Cf. Fig. 1). If, however, tap-water was used instead of distilled water in the preparation of the saline, then at temperatures below 18° C. the diluted blood coagulated more quickly than the undiluted blood, at 10° C. the difference being as great as 24 per cent. This effect appears to be due to some other

property than the calcium (perhaps the alkalinity) of the tap-water; calcium chloride ($\frac{1}{1000}$) dissolved in distilled water saline had no such influence.

(b) *Calcium Chloride.*—Solutions of various strengths were made up in normal saline, and the method given above used, the bath being kept at 38° C. The addition of an equal volume of a solution of calcium chloride containing less than $\frac{1}{1000}$ had no more effect on the blood than the addition of an equal volume of normal saline; in stronger solutions the calcium chloride had an inhibitory effect on the production of fibrin. The results of the experiments are plotted in Fig. 7.

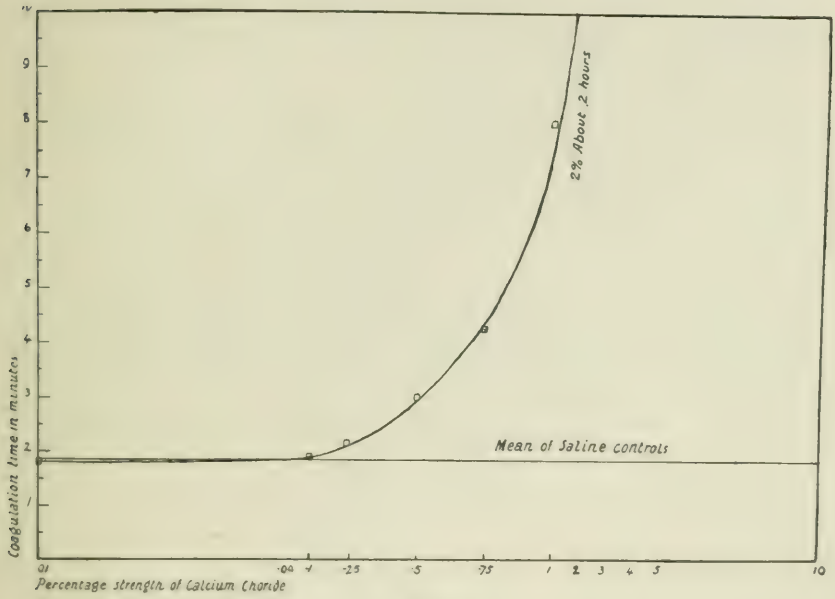


FIG. 7.

(c) *Potassium Oxalate.*—Similar experiments were carried out; an equal volume of $\frac{1}{1000}$ had a slight inhibitory effect on the coagulation rate, $\frac{1.8}{1000}$ delayed the onset of coagulation 60 per cent., and the total amount of fibrin formed was small; a solution of $\frac{1.9}{1000}$ rendered the blood incoagulable.

(d) *Radium Emanation.*—It has been stated that radium has a powerful haemostatic action.* Some experiments were

* Quoted from a Lecture by W. Deane Butcher, reported in "British Medical Journal," Oct. 14th, 1911, p. 894.

therefore made, using a solution of radium emanation in saline. No accelerating effect on the production of fibrin could be detected, but on the contrary there was a slight delay in the onset of coagulation owing to the production of acid in the salt solution.

Conclusions.

1. If due precautions be taken, Dale and Laidlaw's method for determining the coagulation rate of the blood is accurate to within 5 per cent.

2. Daily observations made on a healthy individual show a variation of about 5 per cent. above and below the mean coagulation time for that person.

3. No modification of the blood coagulation rate has been found to be associated with variations in the external temperature, time of day, taking of food, or after moderate exercise.

4. Calcium chloride, when added to healthy blood, does not appear to have any accelerating effect on the coagulation rate.

5. In healthy blood a small proportion of the calcium present is sufficient to bring about coagulation. Decreasing the amount of calcium in solution by the addition of potassium oxalate appears to diminish the amount of fibrin formed, but the delay in the onset of clotting is not affected to the same extent.

THE COAGULATION RATE OF THE BLOOD IN INOPERABLE CANCER.

By ELIZABETH H. LEPPER.

It has been shown that the Coagulometer devised by Dale and Laidlaw is suitable for clinical investigations, and that, provided certain precautions be taken, the results obtained are within about 5 per cent. of the average coagulation time for the person examined.

When investigating the coagulation rate of the blood in cancer patients the following method was adopted: Two observations were made on each case on three different days, and the mean of the six experiments was regarded as the coagulation rate for that person.

In all, 30 female patients were examined; of these, 25 were examined on three different days, 4 on two different days, and 1 only once. As an example one case is recorded in detail.

First Day.

Tube 1, Time of clotting, 102·2 sec.
" 2, " 103·4 " } Mean 102·8 sec. Error 1·1%

Second Day.

Tube 1, Time of clotting, 97·8 sec.
" 2, " 96·2 " } Mean 97 sec. Error 1·6%

Third Day.

Tube 1, Time of clotting, 101·6 sec.
" 2, " 104·4 " } Mean 103 sec. Error 2·7%

Mean of three experiments, 100·9 sec.; mean error, 1·8%; variation between longest and shortest mean, 6 sec.

On six occasions it was not possible to get the mean of two observations.

The average rate of the 30 patients was 94·3 sec. as against a normal mean of 99·3 sec.; the longest time was 105·5 sec., the shortest 82·7 sec.

In Fig. 1 the results obtained are grouped into time-periods, and show the higher percentage of quick rates found in cancer patients. No normal person gave a rate between 80 sec. and 85 sec., whereas two cancer patients did, and between 85 sec. and 90 sec. only one normal as against five cancer cases, the percentage being of course greater still.

The mean error was 3·8 per cent., the greatest 14·2 per cent., the least 0 per cent.

The mean variation between the longest and shortest rate observed was 6·3 sec., the greatest in any one individual being

Coagulation rates of 30 *cancer patients compared with those of* 50 *normal people.*

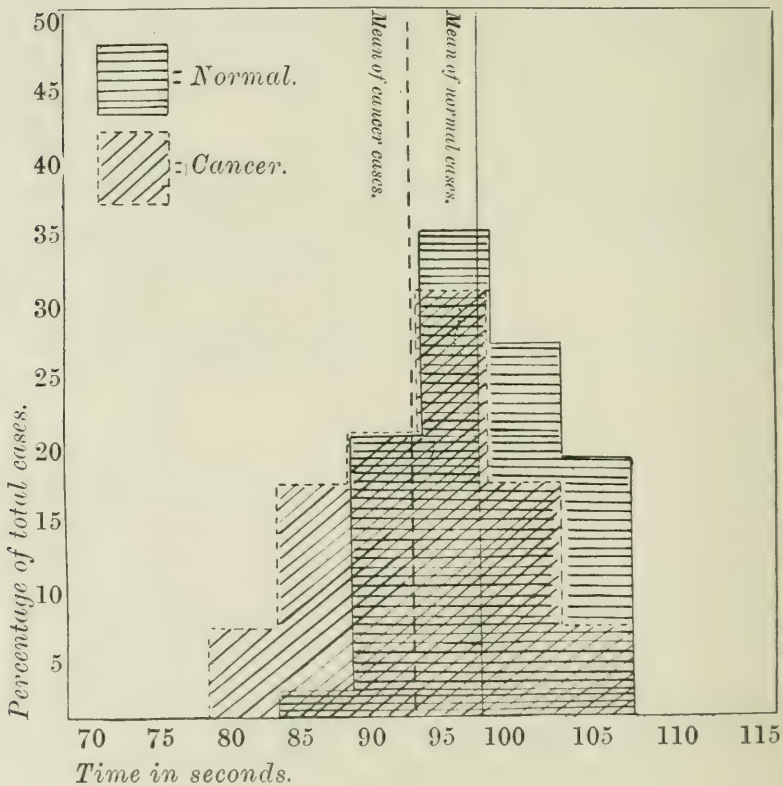


FIG. 1.

18·3 sec., the least 1·5 sec. The details of the three experiments in the last-mentioned most constant result were as follows :—

First Examination.

Tube 1, Coagulation rate, 81·4 sec.
„ 2, „ „ 86·4 „ } Mean 83·9 sec. Error 6%

Second Examination.

Tube 1, Coagulation rate, 86·8 sec.
„ 2, „ „ 84·0 „ } Mean 85·4 sec. Error 3·2%

Third Examination.

Tube 1, Coagulation rate, 84·4 sec.
„ 2, „ „ 84·4 „ } Mean 84·4 sec. Error 0%

Mean of three experiments, 84·2 sec.; mean error, 3 per cent.; variation, 1·5 sec.

The patient who gave the greatest variation in the rate was admitted for intestinal obstruction which was relieved by a cæcal colotomy. At first her general condition was fairly good, but it rapidly became bad, and the second two observations of the coagulation rate showed that the blood clotted much more quickly than at first. Another patient who was getting rapidly worse behaved in a similar manner, but in a third the coagulation rate became slower before death.

It was thought that by grouping the cases it might be possible to determine the common factor responsible for the increased rate of clotting of the blood, which was certainly present in a number of the patients, since 15 of them had rates persistently quicker than 95 sec.

The degree of illness.—Of the 30 cases 14 were extremely ill. These gave a mean rate of 92·5 sec. (slightly quicker than the average rate of all the cases, which was 94·3 sec.).

The shortest time was 82·7 sec., the longest 103·3 sec.; this patient was suffering from carcinoma of the cervix and had uræmic symptoms with general œdema, which possibly accounted for the relatively long coagulation rate.

Eleven cases were fairly well; their average time was 95·3 sec.

Five cases were able to be up and about; these gave an average of 96·2 sec.

In Fig. 2 the high percentage of quick coagulation rates found in patients who were very ill is contrasted with the percentage found in healthy people.

Coagulation rates of cancer patients who were very ill, **14** *cases, compared with normals.*

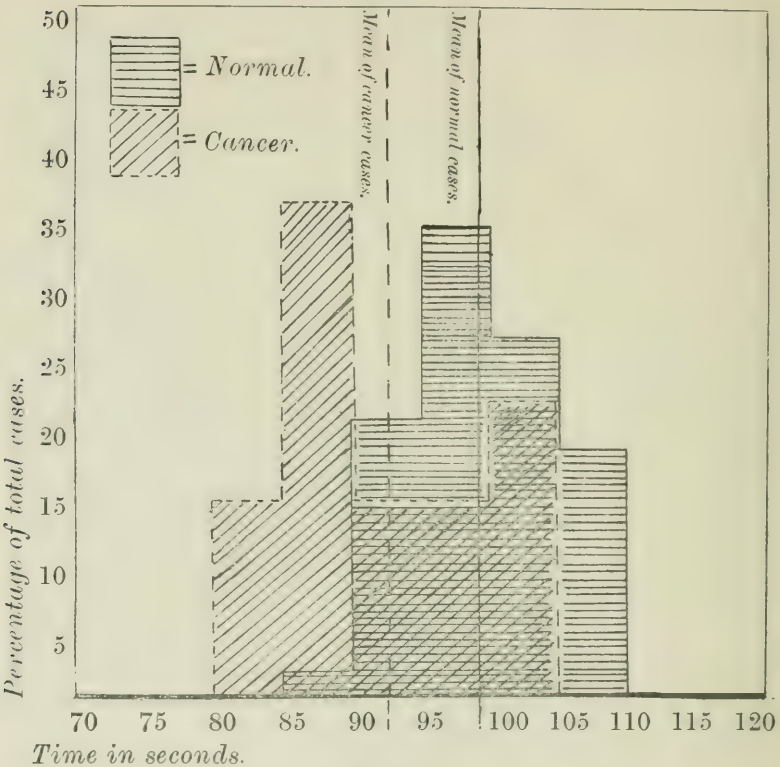


FIG. 2.

From these cases it appears that it is only in the advanced stages of cancer that the coagulation rate is appreciably affected, and it was obviously of importance to find out whether other disease associated with wasting and anæmia also showed an increased coagulability of the blood. Eleven patients who were seriously ill, who were wasted, anæmic, and who had been confined to bed for periods varying from

a fortnight to several months, were examined. Of these, 4 had kidney disease, 1 diabetes, 1 colitis, 2 tuberculous peritonitis, one having a fæcal fistula, 2 heart disease, 1 severe anæmia following hæmorrhage due to fibroids. The average coagulation rate was 99·7 sec., practically the same as the normal mean. The shortest time observed was 82·9 sec.; this occurred in a heart case, and was as rapid a rate as any obtaining in the malignant series. The number of cases having rates shorter than 90 sec. was two, giving a percentage of 18 as against 53 in the cancer patients.

It is, of course, difficult to examine exactly parallel cases, but as far as they go the observations indicate that in cancer the coagulability of the blood is much more frequently increased than in other diseases.

The presence of a large amount of growth.—Thirteen of the cases had a large amount of cancerous material present, either as a primary growth for which no operation had been done, or as secondary deposit in the skin, glands, liver, or pelvis. Only those cases in which the growth could be seen or felt are included in this group.

The average coagulation rate was 93·9 sec., the shortest 82·7 sec., the longest 105·3 sec. The two cases at the extremes appeared to have much the same amount of growth; each had a large mass of glands in the axilla and above the clavicle secondary to carcinoma of the breast. Both had shooting pains down the arm, and marked œdema of the hand. In the patient whose rate was long, the general health was fairly good; she was well nourished, and got up every day; the pain came on in attacks, the œdema increased and diminished from time to time, and the disease did not seem to make much progress. The patient in whom clotting took place quickly was thin; she was never free from pain, the size of the arm increased, and the glands in the anterior triangle became palpable; she had severe shooting pains up the right side of the head, and occasional attacks of severe dyspnœa. Her general condition got worse daily. It therefore appears that alteration of the coagulation rate cannot be correlated with the mass of growth present.

Disintegration of the growth. — Degenerative changes were marked in eight cases, which gave a mean time of

95·1 sec.; in eight cases in which such changes were slight or absent the average rate was 92·3 sec.

In order to exclude degenerative changes, five cases of carcinoma of the breast were examined before operation. The mean rate was 96·8 sec., very slightly quicker than the normal time; but as two cases which clinically resembled carcinoma and at the operation were found to be innocent gave rates of 93 sec. and 97 sec. respectively, no assistance in diagnosis is to be expected from an estimation of the coagulation rate in operable cases.

The presence of fever.—Fever was considered to be present if the temperature rose above 99° F. on more than one occasion during the period that the observations were being made. Seventeen cases are included in this group and gave an average coagulation rate of 92·7 sec. The 13 patients who had no fever gave a mean rate of 96·2 sec. Since temperature *per se* within the limits obtaining in fever has little effect, it would appear that the acceleration noted above depends rather on the conditions occasioning the rise of temperature than on the rise of temperature itself.

Repeated hæmorrhages.—Only three cases had this complication: their rates were 104 sec., 90 sec., and 82·7 sec. The differences are wide, and the cases are too few and the conditions too complicated for any conclusions to be drawn.

Site of the primary growth.—The position of the original tumour does not appear to have any determining effect on the rate of coagulation of the blood. The cases are classified below:—

Site of Primary Growth.	No. of Cases.	Mean Rate of Clotting.
Breast	12 ...	93·5 sec.
Rectum	7 ...	95·4 ,,
Cervix	6 ...	95 ,,
Stomach	1 ...	89·3 ,,
Vulva	1 ...	88·3 ,,
Superior maxilla	1 ...	90 ,,
Rodent ulcer of face	1 ...	96 ,,
Sarcoma of superior maxilla...	1 ...	105·1 ,,

Effect of duration of the disease.—The date of onset was taken from the time at which the patient first noticed a

swelling, or had definite symptoms such as hæmorrhage. The duration varied from 6 months to 12 years. The patient who had sarcoma of the upper jaw said that the swelling dated from a blow on the eye, 14 years previously. Including this case the mean duration was 2·9 years, excluding it 2·5 years. There did not appear to be any relation between the length of time the disease had been in existence and the coagulation rate.

Conclusions.

1. In the cachectic stages of malignant disease the coagulability of the blood is generally, but not always, increased.

2. In most early cases of carcinoma no change in the coagulation rate can be detected; no assistance in diagnosis is therefore to be obtained by an estimation of the coagulation rate.

ON THE FREQUENCY OF THROMBOSIS FOLLOWING LAPAROTOMIES FOR CARCINOMA.

By ELIZABETH H. LEPPER.

The cause of post-operative thrombosis is still obscure; most observers are agreed, however, that carcinoma is a predisposing factor. In order to get some idea of the frequency with which this complication occurs the records of the New Hospital for Women for the years 1901 to 1910 inclusive were examined. The results are recorded in the following table.

It was found that post-operative thrombosis was confined almost entirely to laparotomies, operations on the kidney or for hernia, and amputations of the breast for carcinoma.

Operations.	No.	Cases of Thrombosis.	% Cases of Thrombosis.	Cases of Embolism.	% Cases of Embolism.	% Thrombosis and Embolism.
Laparotomies non-malignant	861	14	1·6	3	0·34	1·9
Laparotomies for cancer ...	281	4	1·4	3	1·06	2·4
For hernia	209	1	0·49	—	—	—
On kidney	70	2	2·8	—	—	—
Amputation of breast, cancer	88	4	4·5	—	—	—
Total Innocent	1,140	17	1·4	3	0·26	1·7
Total Malignant	369	8	2·1	3	0·81	2·9

The greater number of the laparotomies were for diseases of the pelvic organs; but appendicectomies, and operations on the stomach and intestines, were not infrequent.

In the first group of operations, for innocent tumours or inflammatory disease, the 17 cases of thrombosis or embolism were distributed as follows: hysterectomy for fibroids, 8; suspension of the uterus, 3; appendicectomy, 3; salpingo-oophorectomy, 3.

In the second group following operations for cancer the 7 cases of thrombosis or embolism resulted from: hysterectomy, 3; gastro-enterostomy, 1; ovariotomy, 1; excision of tranverse colon, 1; exploratory laparotomy for malignant gall-bladder, 1.

The localisation of the thrombosis following amputation of the breast, which in all four cases was in the veins of the arm on the affected side, suggests that the clot was probably due to some direct injury to the vessel at the time of operation. It was found, however, that in thirty-one cases of excision of tuberculous glands of the neck there was no record of any thrombosis occurring.

Many authors state that in anæmia and cachexia the blood clots more easily than usual. Denk and Hellmann (2), working with a modification of Wright's method, found that there was a distinct increase in the coagulation rate of the blood in cancer patients provided cachexia was present; in the early stages of the disease they found that the acceleration was very slight. Working with the method described by Dale and Laidlaw I have obtained similar results. It is possible that the high percentage of cases of thrombosis which Friedermann (1) found after exploratory laparotomy may have in some measure been due to an increased coagulability of the blood in the inoperable cases.

As regards the causative importance of anæmia, it is difficult to be sure, as it is so often associated with other conditions. In one woman who was anæmic owing to profuse uterine hæmorrhage, the hæmoglobin being 40 per cent., the coagulation rate was 97 sec. (normal average time 100 sec.). In a case of tuberculous broncho-pneumonia complicated by thrombosis in the left internal iliac vein and in the right pulmonary artery, where the hæmoglobin was only 20 per cent., the coagulation rate of the blood was 100 sec.

Severe anæmia does not appear, therefore, to be associated with an increased coagulability of the blood, and if it be a predisposing cause of thrombosis, may perhaps act by leading to degenerative changes in the vessels, the result of malnutrition.

Whipple and Hurwitz (3) have shown that in dogs the administration of chloroform produces marked diminution in the amount of fibrinogen in the blood, followed a few days

later by a return to normal, but in some cases after the initial drop a considerable increase in the percentage of fibrinogen above the normal was noticed.

It was thought worth while to make some observations on patients before and after laparotomy, to see whether any change in the coagulation rate was produced. At first the third, sixth, and tenth days after operation were chosen for making the examination; but, as it was found that the coagulation rate tended to be quicker about the fourteenth day, in the later cases the seventh, fourteenth, and eighteenth to twenty-first were selected.

Eleven patients were examined, of whom six were suffering from cancer. Of these only one, an advanced case of carcinoma of the stomach, showed any marked alteration in the coagulation rate of the blood. Before operation the time taken was 106 sec., on the 10th day 96 sec., the 17th day 86 sec., an increase of about 20 per cent.

Although it is impossible to generalise from so small a number of cases, it does not appear that laparotomy and the associated conditions of anæsthesia, changes in diet, and rest in bed are productive of an increase in the coagulation rate of the blood, except in a small number of cases, and it remains to be determined whether post-operative thrombosis is found in such cases, and whether clotting, when it does occur, is accompanied by an increase in the coagulation rate of the blood.

Conclusions.

Pulmonary embolism occurs more frequently after operation for carcinoma than after operations for other diseases. This is probably to be associated with cachexia, a condition which I have elsewhere shown is liable to be accompanied by an increased coagulability of the blood.

References.

(1) Friedermann, Klinische Erfahrungen über postoperative Thrombosen und Embolien, "Beiträge zur Klinischen Chirurgie," No. 69, 1910.

(2) Denk & Hellmann, Die Verwertung der Coagulationsbestimmung des Blutes in Chirurgie, "Mittheilungen aus den Grenzgebieten der Medizin und Chirurgie," Bd. 20.

(3) Whipple & Hurwitz, "Journal of Experimental Medicine," xiii., No. 1191, 1.

NOTE ON A SARCOMA OF THE BREAST SHOWING EARLY OSSIFICATION.

By HENRY BECKTON.

In this case, as contrasted with those described by Sir John Bland-Sutton in a previous volume of these Archives (Ninth Cancer Report, 1910, p. 98), no special hardness of the growth suggested any degree of calcification in the mass. The tissue was therefore embedded in the ordinary way for paraffin sections, but no difficulty was found in the cutting, the edge of the razor dealing quite satisfactorily with the small calcified trabeculæ met with in the central region of the mass.

Clinical History.—The patient, aged 52 years, had an abscess in her left breast after her first child was born, 27 years ago. She has had five children since, and has suckled all. Five months ago she noticed a lump in her left breast; it gave her no pain, but it has grown since she first noticed it.

On examination a lump is found in the left breast, adherent to the deep fascia, but not to the skin. It is hard and lobulated, and involves practically the whole breast. No glands can be found in the axilla.

Macroscopic Appearances after Removal.—The specimen consists of a large fat breast with axillary glands removed in one mass.

The *Breast* contains a large ovoid mass of new growth (diameters about $2\frac{3}{4}$, 2, and 2 in.), of firm consistency except in its central portion, where hæmorrhage has occurred. No nipple is present; in its region there is a funnel-like depression communicating with the hæmorrhagic centre of the growth.

A few slightly enlarged axillary *lymphatic glands* are present, but whether any of them contains new growth is not macroscopically evident.

Histological Appearances.— *Breast*—The greater part of the section shows only a stroma composed partly of dense fibrous tissue, partly of fine fibrillary tissue enclosing scattered, small, rounded spaces, some of which contain cells. The remainder, however, shows new growth consisting of a mass of cells without alveolar arrangement; a considerable number of these are multinucleated, one such cell showing in one section alone some sixty nuclei. Numerous small spicules of true bone are present in the growth, into which hæmorrhage has taken place.

Glands.—No new growth is present in these.

AN EXPERIMENTAL STUDY OF THE ACTION OF COLEY'S FLUID ON SARCOMATA IN RATS AND MICE.

(Preliminary Communication.)

By E. MUSGRAVE WOODMAN.

In the hands of many clinicians, particularly in America, Coley's Fluid has been found to exert a beneficial influence on a certain proportion of inoperable sarcomata. It does not, however, appear to have any effect on carcinomata.

Experiments were commenced in the endeavour to ascertain the mode of action of these toxins and what influence they may have on transmitted tumours in animals.

The present short paper is in the nature of a preliminary communication, and the work is still in progress. The results of the earlier experiments are given and the conclusions indicated.

At the outset the difficulty of obtaining a suitable tumour was met with. It was necessary to have a growth that could readily be transmitted, but again one which would not grow or degenerate too rapidly. It is imperative that there should be evidence of malignancy, and a large majority of these tumours are found to advance progressively to the death of the animal. Ulceration through the skin occurred frequently, and occasionally the chest wall and lung were invaded. No metastatic deposits have yet been discovered, although white nodules of a parasitic nature have often been found in the liver. In a few cases the tumour became cystic, broke down, and a spontaneous cure was established.

The present experiments fall into three groups:—(*a*) On Healthy Mice; (*b*) Inoculated Mice; (*c*) Inoculated Rats.

(*a*) **Healthy Mice.**—These injections were carried out in order to ascertain the medicinal and the minimal lethal dose in a mouse. Incidentally they proved how harmless the toxins are to normal mice.

In three such mice, an initial dose of $\frac{1}{200}$ ♏ was gradually raised to ♏ v by fractional increments. Similarly in another the dose was increased from $\frac{1}{200}$ ♏ to ♏ x of the undiluted fluid. In two other mice ♏ ij and ♏ iij were given at a primary injection, and to these may be added the case of a small rat, in which an initial dose of ♏ v, followed by a second of ♏ x, was given. In each case the animal appeared entirely unaffected by the injection, although a careful watch was kept for signs of a rigor or of malaise.

As the initial dose of Coley's Fluid for man is ♏ $\frac{1}{4}$ and the smallest fatal injection on record is ♏ ij, the relatively large doses which can be tolerated by healthy mice are remarkable: so much so that the lethal dose was not reached.

(*b*) **Inoculated Mice.**—Two varieties of tumour were made use of. (1) The first was a "mixed-tissue" tumour; that is, a carcinoma with scattered patches of sarcomatous tissue in its substance. In this case the sarcoma was of the spindle-celled variety and represented only a small fraction of the whole, having appeared during the artificial propagation of the original tumour. (2) The second variety was a pure carcinoma. Both tumours were originally obtained from the Imperial Cancer Research Laboratories.

(1) *Mixed-Tissue Tumour.*—A series of twenty-six mice was taken; into each of these a tumour of this variety had been satisfactorily inoculated on the same day. Thirteen of these were injected twice weekly with Coley's Fluid and thirteen were kept as controls. The dose commenced with ♏ j in each case and increased to ♏ iij; in seven animals the injection was made directly into the tumour, while in the remaining six it was introduced into the flank. These tumours all progressed to the death of the animal, and no difference could be detected in the rate of growth between the tumours treated and those used as controls. The animals were unaffected by the injections, and at the subsequent post-mortem examination no difference could be seen.

(2) *Carcinoma.*—A batch of sixteen mice were successfully inoculated on the same date with this tumour. Eight of these were injected with the mixed toxins and eight were similarly treated for purposes of control with normal saline solution. In each case the injection was made into the flank,

Plate I.

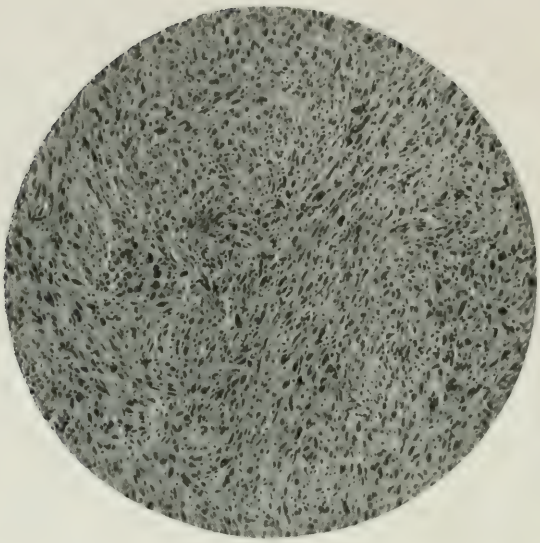


FIG. 1.

Microscopic appearance of rat tumour under $\frac{2}{3}$ in. objective. The growth is seen to be composed of large, elongated spindle cells arranged in whorls or streams crossing the field. Between these, small rounded cells are seen, probably of an inflammatory nature.

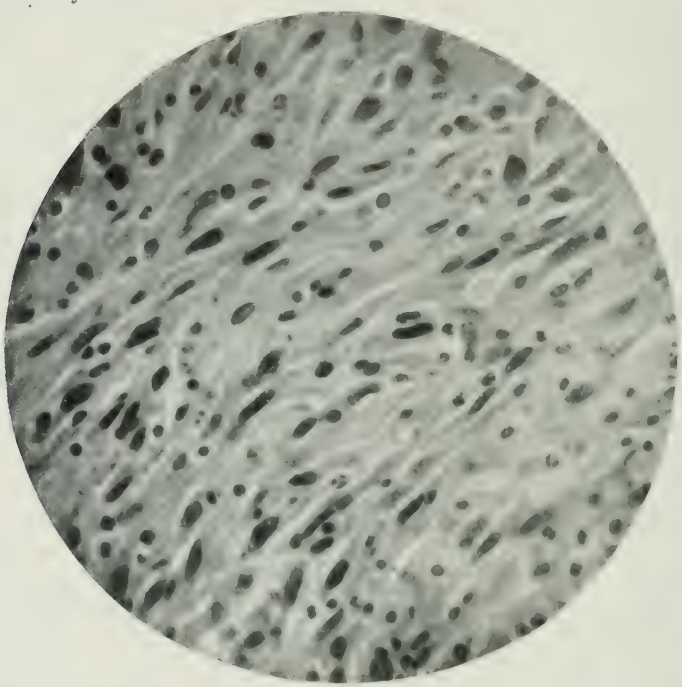


FIG. 2.

The same tumour under a higher power ($\frac{1}{6}$ in. objective). The nuclei are seen to be large, oval, and irregular both in size and shape. Nucleoli and chromatin material are seen, and in places active division is taking place.

and consisted of an initial dose of ♏ ij, rising to ♏ iij. The size of the tumours was measured by callipers, and the shape recorded graphically.

In every instance the growth steadily increased in size, and no difference could be detected between those treated with the toxins and those used as saline controls. At death the tumours had in many cases degenerated and become caseous, but no trace of hæmorrhagic infiltration had occurred.

The potency of the fluid used in Series A and B was tested on the human subject. An initial injection of ♏ ¼ was given to a man suffering from an endosteal sarcoma of the thigh: when an injection of ♏ ij had been reached hæmorrhagic diarrhœa set in and treatment had to be discontinued.

The sterility of the fluid used was tested on two different occasions. Six different media were used, and no growth resulted.

The negative results of these experiments point to the fact that healthy mice are highly tolerant to such injections, and that mice inoculated with tumours that are largely or wholly carcinomatous are similarly unaffected by the treatment.

(*c*) **Inoculated Rats.**—Owing to the difficulty in obtaining a suitable mouse sarcoma, experiments on rats were commenced. A rat with a slowly progressive sarcoma was obtained and the tumour propagated. On histological examination it was seen to consist of elongated spindle cells, arranged in whorls typical of spindle-celled sarcoma, with here and there round cells of inflammatory origin. In sections of the tumour stained for Altmann's granules, Beckton found the larger portion of the growth to be entirely devoid of granules, pointing to its malignant nature. (Plate I., Figs. 1 and 2.)

These experiments are as yet uncompleted, but the results at present are encouraging.

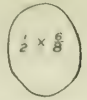
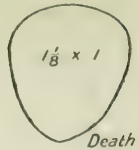
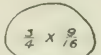
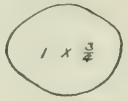
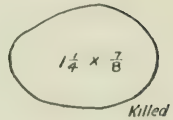
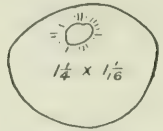
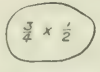
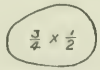
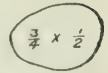
In the first series ten rats with tumours were taken, five of these being inoculated with Coley's Fluid and five with saline solution (·9 per cent.), to be used as controls. The results of treatment are given in the accompanying Table.

SERIES A. COLEYS FLUID INJECTIONS. Mar. 20TH — May 15TH 1912

	MAR 20	MAR 27.	AP 4.	AP. 10	AP. 23	AP. 30	MAY 1ST 7TH–15TH
1. Female rat	$\frac{7}{8}$" × $\frac{5}{8}$" ηι II injected	Scab left tumour gone	– (nil)	–	–	–	–
2. Female rat	$\frac{5}{8}$" × $\frac{1}{2}$" ; 1" × $\frac{3}{4}$" ηι IV injected.	$\frac{5}{16}$" × $\frac{1}{2}$" ; $\frac{3}{4}$" × $\frac{15}{16}$" Deep ulcerations in both tumours ηι III into belly.	$\frac{3}{8}$" × $\frac{3}{8}$" ; $\frac{3}{4}$" × $\frac{1}{2}$" ηι III into belly	Scab ; $\frac{3}{8}$" × $\frac{1}{2}$" soft effluent. ηι III into belly	P.M R dried scab no trace of Tumour. L Stinking abscess cavity No trace of growth		—
3. Male rat	MAR. 20	MAR. 27	AP. 4	AP. 10	AP. 23	AP. 30.	MAY 1ST – 7TH – 15TH
	$\frac{7}{8}$" × $\frac{6}{8}$" ηι III	scab left. Tumour entirely gone	–	–	–	–	–
4. Female rat	MAR. 20	MAR. 27	AP. 4	AP. 10.	AP. 23.	AP. 30.	
	$\frac{6}{8}$" × $\frac{6}{8}$" ; $\frac{1}{2}$" × $\frac{1}{2}$" ηι II	$\frac{5}{8}$" × $\frac{5}{8}$" ; $\frac{1}{2}$" × $\frac{3}{8}$" Tumour sloughing rapidly, ηι II into belly.	$\frac{1}{2}$" × $\frac{3}{8}$" ; $\frac{3}{8}$" × $\frac{3}{8}$" ηι III belly	$\frac{1}{2}$" × $\frac{3}{4}$" ; $\frac{3}{8}$" × $\frac{3}{8}$" ηι III belly. Tumour adherent to skin	$\frac{1}{4}$" × $\frac{1}{4}$" ηι III into belly	— Tumour entirely gone	—
5. Female rat	MAR. 20	Death MAR. 20 P.M Hæmorrhage into tumour.					
	$\frac{3}{4}$" × $\frac{5}{8}$" ηι III						

In all cases Injection made into Tumour unless otherwise stated

SERIES A — SALINE CONTROLS — Mar 20th - May 17th 1912

	MAR 20	MAR 27	AP 4	AP 20	AP 17	AP 23	MAY 1 - 7 - 15
№6 Female rat	$\frac{1}{2} \times \frac{3}{8}$  ηγ V Saline	$\frac{1}{2} \times \frac{6}{8}$  ηγ V Saline	$1 \times \frac{7}{8}$  ηγ V Saline	$1\frac{1}{8} \times 1$  Death			
№7 Male rat	$\frac{1}{2} \times \frac{3}{8}$  ηγ V Saline	$\frac{3}{4} \times \frac{9}{16}$  ηγ V Saline	$1 \times \frac{3}{4}$  ηγ V Saline	$1\frac{1}{4} \times \frac{7}{8}$  Killed			
№8 Female rat	$\frac{1}{8} \times \frac{1}{8}$  ηγ V Saline	$\frac{6}{8} \times \frac{7}{8}$  ulceration present ηγ V Saline	$1\frac{1}{4} \times 1\frac{1}{16}$  ηγ V Saline ulceration present	$1\frac{1}{2} \times 1\frac{1}{4}$  Killed			
№9 Male rat	$\frac{3}{4} \times \frac{1}{2}$  ηγ V Saline	$\frac{3}{4} \times \frac{1}{2}$  ηγ V Saline	$\frac{3}{4} \times \frac{1}{2}$  ηγ V Saline	$\frac{3}{4} \times \frac{1}{2}$  ηγ V Saline	$\frac{3}{8} \times \frac{1}{4}$  ηγ V Saline	–	–
№10 Male rat	$\frac{1}{4} \times \frac{1}{4}$  ηγ V Saline	$\frac{1}{4} \times \frac{1}{4}$  ηγ V Saline	$\frac{1}{4} \times \frac{1}{4}$  ηγ V Saline	$\frac{1}{8}$ ηγ V Saline	AP. 17 —	AP 23 –	–

Of these rats (see Table) the tumours in Nos. 1 and 3 disappeared after the first injection, while those in Nos. 2 and 4 proved more resistant, but in both cases the tumour eventually disappeared, though No. 2 died of secondary (septic) infection. No. 5 rat died within an hour of injection, and on post-mortem examination a severe recent hæmorrhage had taken place into the centre of the tumour, although no hæmorrhage had occurred elsewhere in the body. Of the control tumours, those in Nos. 6, 7, and 8 progressed until the rat died or had to be killed, while No. 9 tumour remained for a time and then became atrophic. No. 10 similarly remained stationary for three weeks and then disappeared. In these two latter cases the rats were rather large and rough-haired and not suitable for the purpose. In the rats of this series, injection took place into the tumour itself, unless ulceration were present. It is known that direct injection in the human subject is more rapid and certain in result and more economical of material. In subsequent injections I have, however, inserted the fluid into the chest wall at least 1″ away from the tumour.

In a series just commenced another rat died immediately after injection. At the time of treatment the tumour measured $\frac{12}{16}'' \times \frac{10}{16}''$ in diameter and was of firm consistency. The animal received ♏ iij of Coley's Fluid into the left side, but died half an hour later. At the autopsy a large hæmorrhage was seen in the centre of the tumour, although the injection had not been made locally. A careful examination revealed no other focus of hæmorrhage, and beyond a slight local reddening the site of injection was not affected. (Plate II.)

These two fatal cases, both accompanied by hæmorrhage into the tumour, are remarkable, and accord with the clinical facts seen in man. In neither case was the dose excessive, and in both the resultant lesion was the same. The suggestion that the drug acted as a local irritant cannot arise in the latter case, as injection was not made into the tumour itself. The inference is therefore that the toxins exert a selective influence on the tumour, but the subject needs further investigation.

Plate II.

Fig. 3.

Naked-eye appearance of a tumour (Series B) on section. The dark opaque area represents an extensive hæmorrhage into the substance of the tumour, while the lighter parts towards the periphery represent tumour substance.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